Anti-proliferative and antimigratory potential of cinnamomum cassia bark extract on breast cancer cells

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ABSTRACT:

Background: Breast cancer is the most prevalent cancer in women in the world, accounting for approximately 25% of all female malignancies, with a higher frequency in developed countries. Breast cancer develops when abnormal cells in the breast divide and multiply, turning it into a malignant tumor. Cinnamic acid derivatives have been shown to suppress breast cancer cells and also suppress proliferation and tumor progression.

Aim: The aim of the study is to assess the Anti- proliferative and antimigratory potential of cinnamomum cassia bark extract on breast cancer
cells.

Materials and methods: Estrogen-dependent (MCF-7) breast cancer cell lines were received from the NCCS, Pune, for cell line maintenance. Then, the Herbal Extract Preparation and Cell Viability (MTT) Assay were evaluated. Mcf7 cells (2×105 cells/well) were seeded into six-well culture plates for a morphology study , and cell migration examined using a scratch wound healing assay. A 200-l tip was used to scrape the cell monolayer in order to make a wound, and pictures of the results were taken. Then, Statistical analysis- All data obtained were analyzed by One Way ANOVA followed by SPSS.

Results: In our study Cells were treated with C.cassia bark extract 20 µg/ml for 24 h along with control group. These results revealed that C.cassia bark inhibited the proliferation of MCF-7 cells, changed the cytoplasmic morphology, promoted the apoptosis of MCF-7 cells, reduced the invasion and migration ability of MCF-7 cells, and exhibited anti-breast cancer effects. Therefore C.cassia bark primary constituents are linked to apoptosis, invasion, and metastatic targets in breast cancer cells. Overall the results of this study suggest that c.cassia extract could be employed as a possible anti- tumor agent.

Conclusion: As a result of the findings, Cinnamomum cassia was discovered to be cytotoxic to breast (MCF-7) cancer cell lines and also inhibits migration of breast cancer cells .Further invivo investigations and clinical trials

may be conducted in order to develop c.cassia extract into a useful medication for treating breast cancer cells and make it commercially available.

Keywords: C.cassia bark, Breast cancer, MCF-7, Proliferation, Anti-migration.

INTRODUCTION:

The world's biggest cause of death and illness is still cancer. Around 10 million newly diagnosed cancer cases are recorded each year, 6 million of which result in mortality worldwide (1,2). The most prevalent cancer and the leading cause of cancer-related death in women worldwide are breast cancers (3). Breast cancer is the most prevalent cancer in women in the world, accounting for approximately 25% of all female malignancies, with a higher frequency in developed countries (4). Breast cancer develops when cells in your breast grow and divide in an uncontrolled way, creating a mass of tissue called a tumor (5).

Plethora of medicinal plants have attracted attention among the scientific communities for its therapeutic efficacies against a number of diseases including cancer (6–14,14–16). Cassia cinnamomum bark is a Lauraceae family that is commonly used in traditional Chinese medicine. The plant's constituents cinnamaldehyde, cinnamic acid, cinnamate, and a number of others have been discovered to possess strong anticancer, anti-inflammatory, antibacterial and cardioprotective qualities (17). And also it is frequently used to treat ailments like dyspepsia, gastritis, blood circulation issues, and inflammatory disorders (18). The bioactive ingredient in cinnamon, cinnamaldehyde, has been found to inhibit the growth of a proliferation of human cancer cell lines, including breast, leukemia, ovarian, and lung tumor cells (19–22). In a preliminary study, Hep G2 cells exposed to C. cassia extract showed anti-proliferative action (23). In previous studies showed that compared to the control cells, SiHa cells treated with Cinnamomum water extract showed less colony development (24).

Even though the alpha melanocyte-stimulating hormone induces melanin synthesis, the essential oils from C. cassia inhibit it, decreasing oxidative stress in murine B16 melanoma cells (25).
Many cinnamaldehyde-derived compounds have been created throughout the years, and they exhibit a range of biological actions in conditions like cancer, diabetes, neuropathy, and cardiovascular disease. Procyanidins and catechins are constituents found in cinnamon bark.

Chemotherapy is a common kind of cancer treatment, although it is hindered by unfavorable toxic side effects and drug resistance (26). Natural plants have historically been used to cure a variety of illnesses, including cancer, and their scientific isolation and characterisation for development into cancer-fighting pharmaceuticals emphasizes their significance (6). Therefore, the aim of the study is to determine whether cinnamomum cassia bark extract has any anti-proliferative and anti-migratory effects on breast cancer cells.

MATERIALS AND METHODS:

Reagents
DMEM (Dulbecco's Modified Eagle Medium), Phosphate Buffered Saline

(PBS), Trypsin-EDTA, Fetal bovine serum (FBS), were purchased from Gibco, Canada. Acridine orange (AO), ethidium bromide (EtBr), Dimethyl sulfoxide (DMSO), [3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT), DAPI, AO/EtBr were purchased from Sigma Chemical Pvt Ltd, USA. All other chemicals used were extra pure of molecular grade and were purchased from SRL, India.

Cell line maintenance

Estrogen dependent (MCF-7) breast cancer cell lines were obtained from the NCCS, Pune. The cells were grown in T25 culture flasks containing DMEM supplemented with 10% FBS and 1% antibiotics. Cells were maintained at 37◦C in a humidified atmosphere containing 5% CO2. Upon reaching confluency, the cells were trypsinized and passaged.

Preparation of the Herbal Extract

Bark powder of Cinnamomum Cassia obtained from IMPCOPS (Chennai, India) was used for the present study. About 50g of (Cinnamomum Cassia Bark) powder was soaked in 500 mL of 95% ethanol and kept at room temperature for 3 days in a static condition. Then the solution was filtered with crude filter paper followed by whatmann paper. Fine filtrate was subjected to rota evaporation after that 3g of the material was obtained. The total ethanol extract was concentrated in a vacuum evaporate and immediately stored at 4˚C.

Cell viability (MTT) assay

The cell viability of plant extract treated MCF-7 cells was assessed by MTT assay. The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. MCF-7 cells were plated in 96 well plates at a concentration of 5x103 cells/well 24 hours after plating, cells were washed twice with 100µl of serum-free medium and starved by incubating the cells in serum-free medium for 3 hours at 37ºC. After starvation, cells were treated with Cinnamomum Cassia Bark extract different concentrations for 24 hours. At the end of treatment, the medium from control and Cinnamomum Cassia Bark extract treated cells were discarded and 100µl of MTT containing DMEM (0.5 mg/ml) was added to each well. The cells were then incubated for 4 h at 37ºC in the CO2 incubator.

The MTT containing medium was then discarded and the cells were washed with 1x PBS. Then the formazan crystals formed were dissolved in dimethyl sulfoxide (100µl) and incubated in dark for an hour. Then the intensity of the color developed was assayed using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in the control medium without any treatment was represented as 100%. The cell viability is calculated using the formula: % cell viability = [MCF-7 nm of treated cells/MCF-7 nm of control cells]×100.

Morphology study

Based on MTT assay we selected the optimal doses (IC-50: 20µg/ml) for further studies. Analysis of cell morphology changes by a phase contrast microscope. 2×105 cells were seeded in 6 well plates and treated with Cinnamomum Cassia Bark extract (concentration for MCF-7 cells) for 24h. At the end of the incubation period, the medium was removed and cells were washed once with a phosphate buffer saline

(PBS pH 7.4). The plates were observed under a phase contrast microscope.

Cell migration analyzed by scratch wound healing assay

Human breast cancer cell lines (2x10' cells/well) were seeded onto six-well culture plates. The cell monolayer was scratched using a 200ul tip to create a wound. The detached cells were removed by washing with IX PBS and adding fresh culture medium with (20µg/ml of Cinnamomum Cassia Bark extract) for 24 h along with control group for 24 h. After incubation, the wells were washed and fixed in 4%paraformaldehyde. Photographs were taken using an inverted microscope (Euromex, The Netherlands).

Statistical analysis

All data obtained were analyzed by One way ANOVA followed by Student's-t-test using SPSS. represented as mean ± SD for triplicates. The level of statistical significance was set at $p<0.05$.

RESULTS:

Effect of Cinnamomum Cassia Bark extract on cell viability of breast cancer cell line

The cytotoxic potential of Cinnamomum Cassia Bark extract in breast cancer cells was assessed by MTT assay. The cells were treated with different concentrations (5-50µg/ml) of Cinnamomum Cassia Bark extract for 24h. Cinnamomum Cassia Bark extract treatment significantly decreased the viability of MCF-7 cancer cells compared to control at 24h time point (Figure-1). The percentage of cell viability reduced gradually with increase in the concentration. We observed the 50% growth inhibition at (20µg/ml) concentration. Hence, IC50 dose (20µg/ml) was considered for the further experiments.

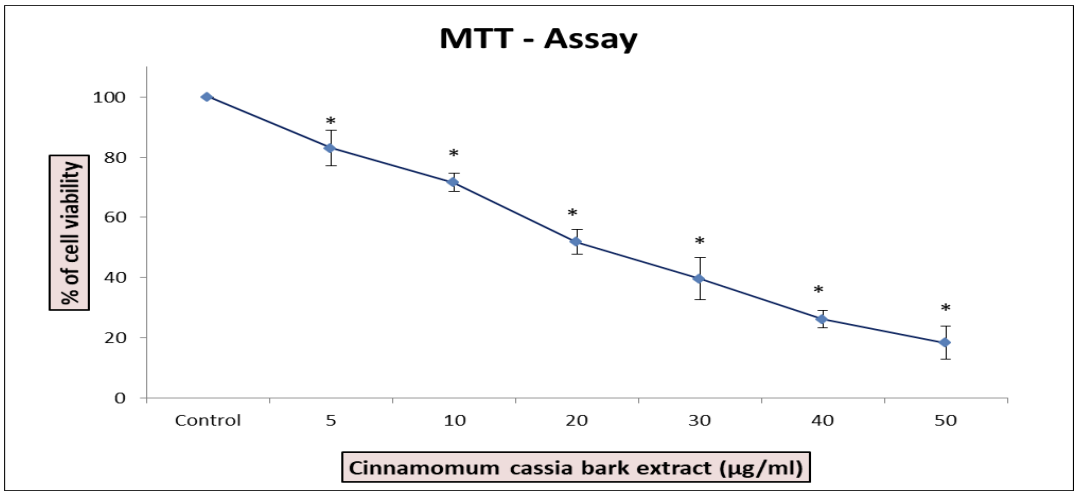

Figure 1: The cytotoxic effects of c.cassia bark on breast cancer cells. Cells were treated with (5, 10,20, 30,40 and 50µg) for 24 h, and cell viability was evaluated by MTT assay. Data are shown as means ± SD (n = 3). * compared with the control blank group, $p < 0.05$.

The effect of c.cassia bark on cell morphology

The cell morphological analysis of c.cassia bark extract treated breast cancer cells was observed in an inverted phase contrast microscope. The MCF-7 cells were treated with c.cassia bark extract (20µg/ml) for 24

h, compared with the untreated cells, treated cells showed significant morphological changes, which are characteristic of apoptotic cells, such as cell shrinkage and reduced cell density were observed in the c.cassia bark extract treated cells (Fig.2). Cells undergoing apoptosis also displayed other types of morphological changes such as rounded up cells that shrink and lose contact with neighboring cells. Some sensitive cells were even detached from the surface of the plates.

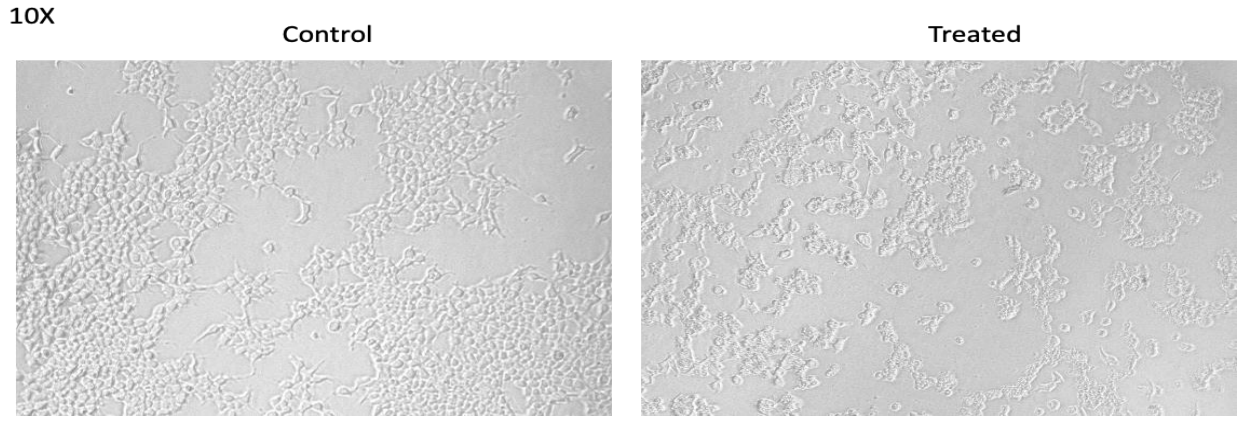

Figure 2: Cells were treated with C.cassia bark extract 20µg/ml for 24 h along with the control group. Images were obtained using an inverted Phase contrast microscope.

Inhibitory effects of c.cassia bark extract on cell migration potential of breast cancer cells.

To check the anti-migratory and invasive potential of c.cassia bark extract, we performed scratch wound healing assay in breast cancer cells. A wound was created by scratching cell monolayers of and treated with c.cassia bark extract for 24h. Non-treated cells repaired the wounded area within 24h of incubation,whereas c.cassia bark extract treatment significantly reduced the wound healing compared to control (Figure. 3). From this experiment, it is clear that c.cassia bark extract inhibits migrative potential of MCF-7 cells.

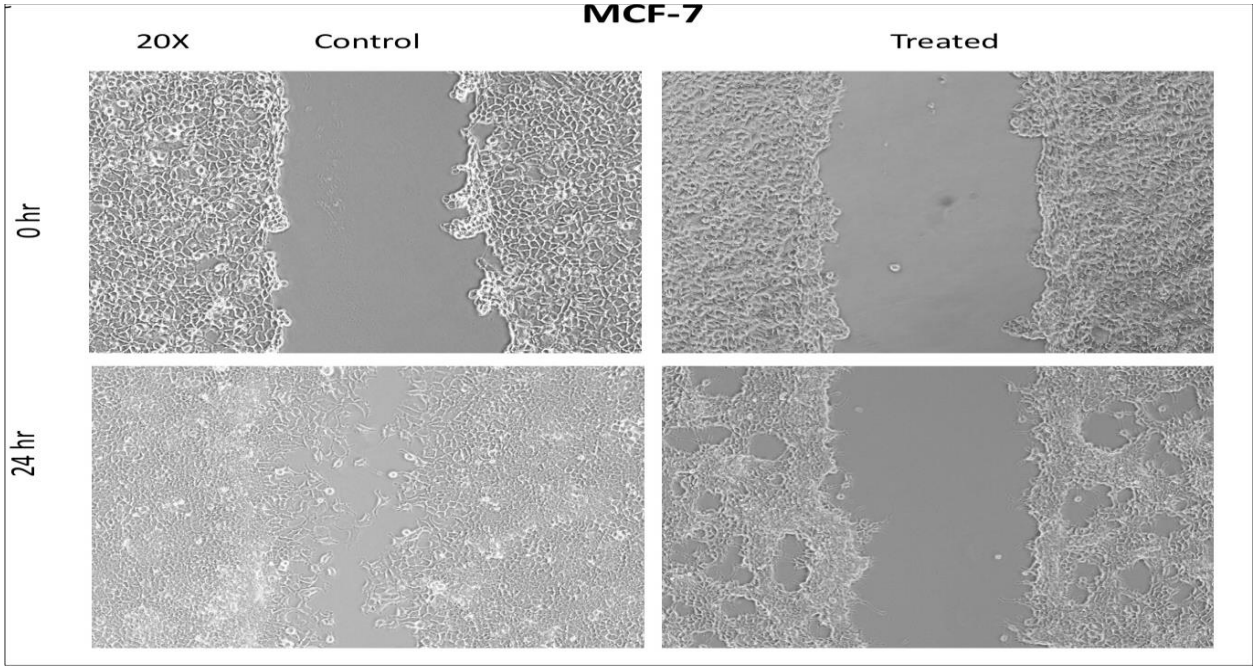

Figure 3: Cells were treated with C.cassia bark extract 20µg/ml for 24h along with the control group. Images were obtained using an inverted Phase contrast microscope.

DISCUSSION:

In this study, fractions of C.cassia bark were screened for their ability to inhibit apoptosis, anti-proliferation and anti-migration activity in (MCF-7) human breast cancer cell lines. The presence of chemical constituents like terpenoids, Phenylpropanoids, Glycosides, lignanoids, Cinnamaldehyde and other compounds found in the c. cassia extract may have tumor growth inhibitory effects. Procyanidins and catechins, the two primary components of cinnamon, are most directly associated with the apoptosis, invasion, and metastasis of breast cancer cells.

Our team has extensive knowledge and research experience that has translate into high quality publications (27–36). In previous investigations, the components of C. cassia have been thoroughly investigated in relation to head and neck squamous cell carcinoma, oral cancer, cervical cancer, and lung cancer. According to Brigitta et al., populus nigrum extract induced antioxidant activity, anticancer capabilities on a breast cancer cell line, an antiangiogenic effect, and an immunomodulatory potential on human primary dendritic cells. Ohnuma et al. looked into the probable ways by which procyanidins (0-300µg/mL), bioactive components of C. cassia, can inhibit lung cancer cell lines A549, LK-2, and LU-99. Additionally, C. cassia extracts lessen the invasiveness and motility of tumor cells brought on by transforming growth factor as well as the growth of human lung adenocarcinoma tumors (TGF). It may reduce the viability of cancer cells, impede the cell cycle, cause apoptosis, oxidative stress, and DNA damage.

Several earlier studies reported that plant derived compounds inhibit cell proliferation and cell migration in a variety of cancer cells (6,37–41). According to studies by Pasupuleti et al., the procyanidin component of cinnamon and its aqueous extract both diminish the activity of the VEGFR2 kinase, which in turn reduces the cancer-related angiogenesis. In 2013, Kin et al. discovered that procyanidin C1, which was derived from the bark of C. cassia, may inhibit TGF-induced EMT and cell metastasis in A549 lung cancer cells. Despite the fact that research by Benjaporn et al. demonstrated that bisphosphonates directly inhibit the growth and migration of MCF-7 cells. The results indicate that ROS production, DNA damage, and mitochondrial malfunction are principally responsible for BP's antiproliferative effects and apoptosis in MCF-7 cells. Cinnamic aldehyde inhibits tumor necrosis factor alpha (TNF-α)-induced interleukin-8 (IL-8) production and NF-kB activity in A375 cells .

Therefore, in the current study, our cell experiments showed that C. cassia bark extract inhibited the proliferation of MCF-7 cells, changed the cytoplasmic morphology, promoted the apoptosis of MCF-7 cells, reduced the invasion and migration ability of MCF-7 cells and exhibited anti-breast cancer effects. However, our findings indicated that C. cassia bark extract was the most effective extract, with an IC50 value of 20µg/mL in the MCF-7 cell line, which was in line with the previous study by Mohammed et al., in which R. stricta fruit ethyl acetate extract

(RSF EtOAc) had an IC50 value of 27μg/mL in the MDA-MB-231 cell line.

CONCLUSION:

As a result of the findings, Cinnamomum cassia was discovered to be cytotoxic to breast (MCF-7) cancer cell lines. The chemical constituents found in the extract reveals the anticancer agents of the extract as it suppresses the migration and proliferation of MCF-7 cells, the results of this study suggest that c.cassia extract could be employed as a possible anti-tumor agent. Further *in vivo* investigations and pre-clinical trials may be conducted in order to develop c.cassia extract into a useful medication for treating breast cancer cells and make it commercially available.

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