



Evaluation of *In vitro* Anticancer Activity of Poly Herbal Extract (*Vitis vinifera*, *Nigella sativa* and *Curcubita pepo* Seeds)

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ABSTRACT

Cancer is a group of diseases involving abnormal cell growth in the body. There are several types of cancer such as breast cancer, lung cancer, kidney cancer. It can grow anywhere in the body which is made up of trillions of cells. To evaluate the *in vitro* anticancer activity of poly herbal extracts of seeds (*Vitis vinifera*, *Nigella sativa* and *Curcubita pepo*). To study the anticancer activity of poly herbal extract of *Curcubita pepo* (pumpkin seeds) *Vitis vinifera* (grape seeds) and *Nigella sativa* (black cumin) seeds by using methanolic extract. Grape seeds, *Curcubita pepo*, *Nigella sativa* was collected from the herbal medical shop. The seed was extracted to find the cytotoxic active constituents using methanol by maceration technique. The seeds was filtered and distillation made by using the seed extract and the cancer cell line studies were carried out. The poly-herbal formulation to evaluate cytotoxic activity showed good results in breast cell lines. It was found that the seeds have the anti-cancer activity.

Keywords: Anti-cancer; Poly herbal extract; Cell line; MTT assay; Black cumin

INTRODUCTION

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. These contrast with benign tumours, which do not spread. In terms of National Cancer Institute (NIT), cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancer can start anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. When cancer develops, however, this orderly process breaks down. As cells become more and more abnormal old or damaged cells survive when they should die, and new cells form when they are not needed. These extra cells can divide without stopping and may form growths called tumours [1-3].

Pathophysiology of breast cancer diagram

Breast cancer is a malignant tumour that starts in the cells of the breast. Like other cancers, there are several factors that can raise the risk of getting breast cancer. Damage to the DNA and genetic mutations can lead to breast cancer have being experimentally linked to oestrogen exposure. Some individual inherit defects in the DNA and genes like the *BRCA1*, *BRCA2* and *P53* among others. Those with a family history of ovarian or breast cancer thus are at an increased risk of breast cancer. The immune system normally seeks out cancer cells and cells with damage DNA and destroys them. Breast cancer may be a result of failure of such an effective immune defence and surveillance. These are several signalling systems of growth factors and other mediators that interact between stromal cells and epithelial cells. Disrupting these may lead breast cancer as well [4].

Treatments

Chemotherapy: These drugs are a main treatment for breast cancer. Side effects are likely with chemotherapy or chemo because of the way it works. Chemo kills or slows the growth of

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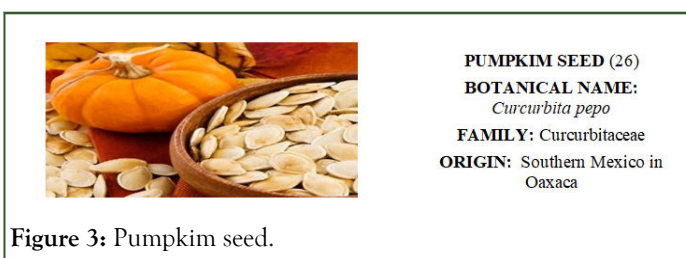
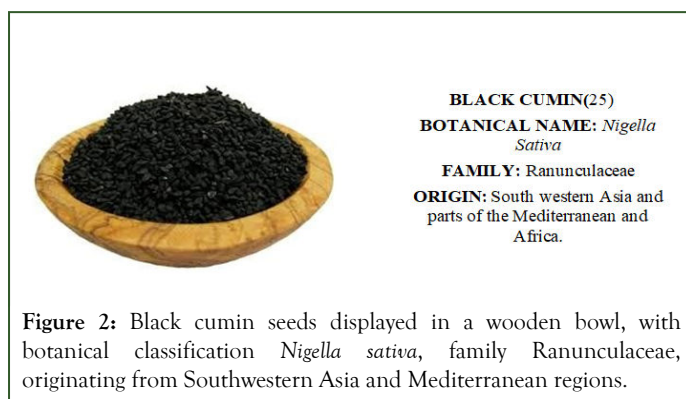
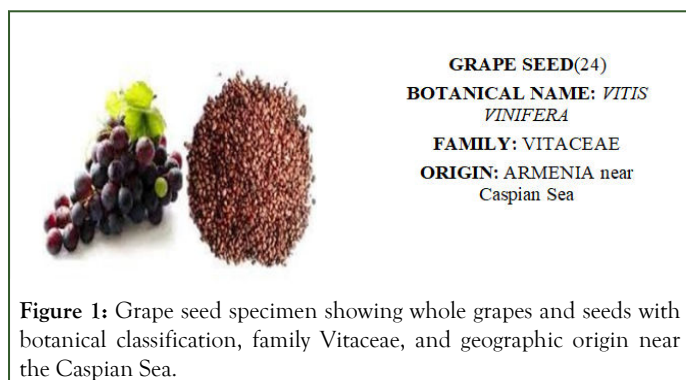
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rapidly multiplying cells. This includes both cancer cells and some normal cells in your body.

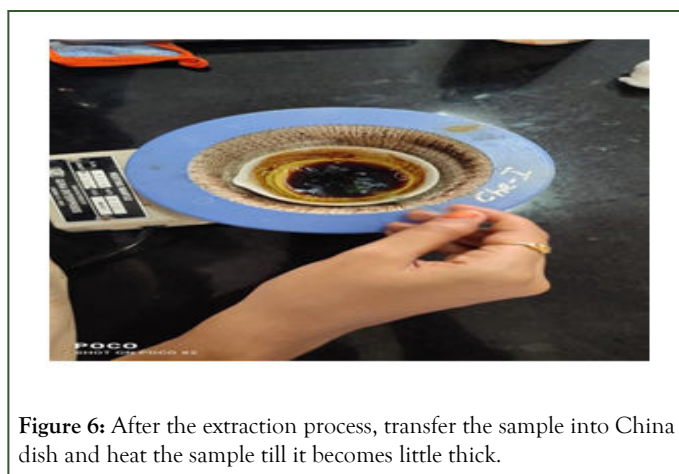
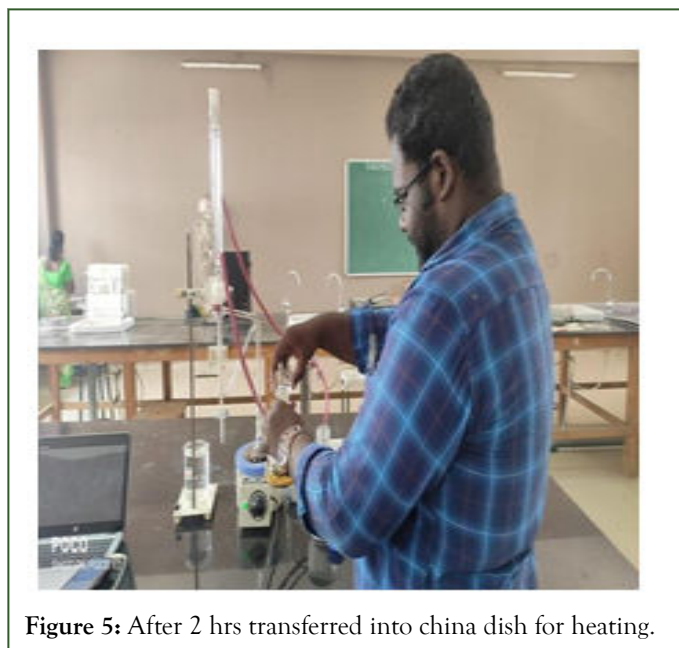
Hormone therapy: This class of drugs works on breast cancers with hormone receptors. Female hormones-estrogen and progesterone stimulate growth of hormone receptor-positive breast cancer cells [5]. Hormone therapy works to lower hormone levels or block the hormone receptor on the cancer cells.

Aromatase inhibitors: These are a type of hormone therapy for breast cancer. They lower hormone levels blocking an enzyme (aromatase) necessary for making estrogen. Hormone therapy side effects include hot flashes, fatigue, vaginal dryness and night sweats (Figures 1-3).



MATERIALS AND METHODS

Petridish, beaker, methanol, small dish, soxhlet extractor, filter paper. The methods were conducted in the following steps (Figures 4-8).



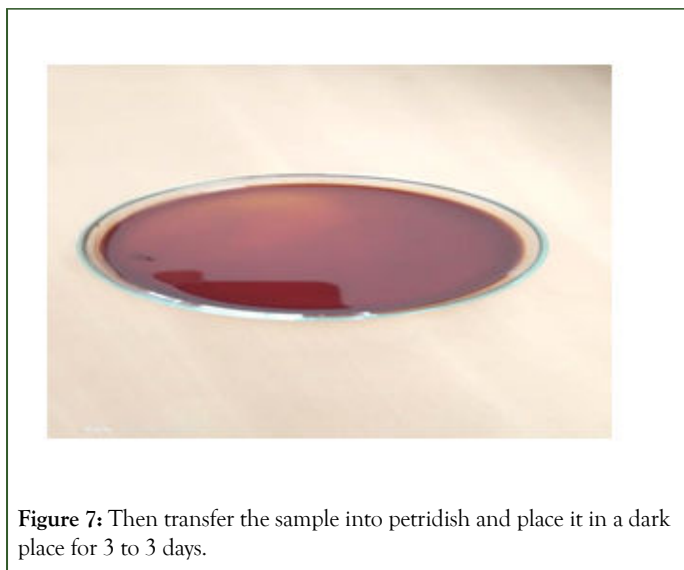


Figure 7: Then transfer the sample into petridish and place it in a dark place for 3 to 3 days.



Figure 8: After two days the sample was given for breast cell line studies.

RESULTS AND DISCUSSION

Collection of seeds

We collected the seeds of *Vitis vinifera*, *Nigella sativa*, and *Curcubita pepo* in the value of 8 grams. Dried the seeds for 2 days. Then weighed 8 grams of seeds and soaked them in 300 ml of methanolic extract. Stored them for 14 days to get the active constituents.

Extraction of seeds maturation techniques

After completion of 14 days, the soaked seeds was preceded to extraction process. So that we will get the active constituents separately. Boiling point of methanol is 64.7°C. The extraction

process was done under 48°C for 10 minutes and slightly increased the value for every 10 minutes for 55°C, 60°C, 65°C and upto 70°C was done. After that, transferred the sample into china dish and boiled using heating mantle for 15 minutes. Then it will become little thick [6]. Then transfer it into petridish. Keep aside for 2 days. So that you will get the active constituents. After getting the active constituents, the sample was proceeded to breast cell line studies.

Cell line anti-cancer activity MTT assay

The cell line was performed in Drug Testing Laboratory Eachanari, Coimbatore. The cell line was received from NCC, Pune. A549 is established and well characterized cell line that has demonstrated reproducible results (Figure 9). The culture medium used in the cell is MEM-medium supplemented with fetal Bovine serum. The assay method MTT was performed (Table 1). The reagents MTT solution (1 mg/ml), DMSO (100%), PBS (pH 7.2) was used. The plates were incubated at 37°C with 5% CO₂ for 3-4 hrs at 570 nm. The imaging was done by phase contrast microscope [7-9].

Report

Anticancer activity - MTT cell line: A549

Sample particulars: Seeds extract

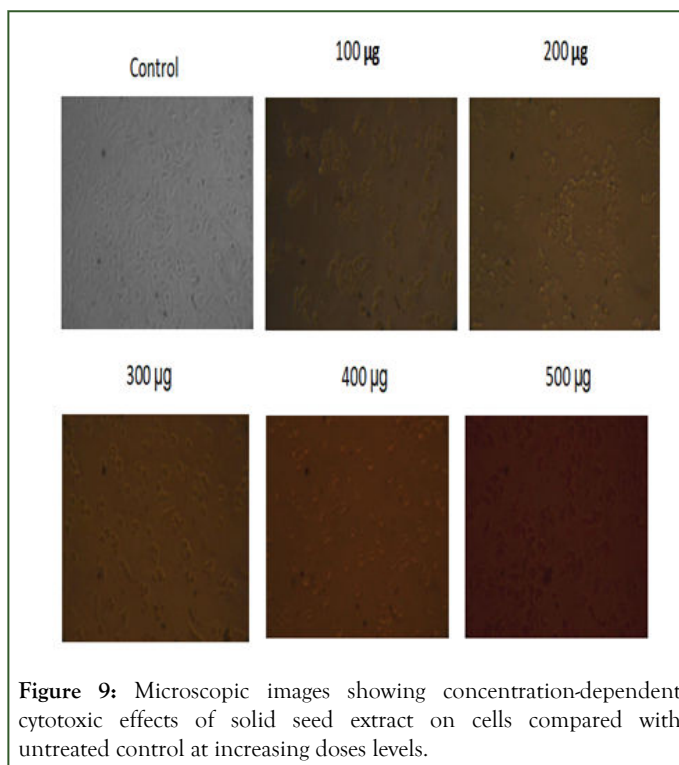


Figure 9: Microscopic images showing concentration-dependent cytotoxic effects of solid seed extract on cells compared with untreated control at increasing doses levels.

Table 1: Cytotoxicity of solid seed extract B2100039-1 across concentrations (100–500 µg), showing decreased cell viability and mild to moderate reactivity.

Concentration µg	B2100039-1 Solid seed extract		
	Cytotoxicity	Cell viability	Reactivity
100	33.4	66.6	Mild
200	54.8	45.2	Moderate
300	57.1	42.9	Moderate
400	57.2	42.8	Moderate
500	58.1	41.9	Moderate

CONCLUSION

Breast cancer is one of important cancers among women which is known as the second most common cancer worldwide. Diagnosis of breast cancer is main aspects of breast cancer therapy. There are many side effects in allopathic medicines. So we tried for the poly-herbal formulation to evaluate cytotoxic activity. In this we have got good result in breast cancer cell line. Hence we conclude that our seeds have anticancer activity. Extending current knowledge into actual prevention awaits further studies from a range of aspects including methodology, individual and collaborative research environment, and ethics.

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