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Original Research Article

# Antiproliferative effects of *Vanilla planifolia* leaf extract against breast cancer MCF-7 cells

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## ABSTRACT

**Background:** Breast cancer is the most common cancer disease among females in India and worldwide. This needs a critical research for finding the drugs to treat breast cancer with less side effects. The aim of the present study is to reveal the anti-proliferative effects of vanilla extract against MCF-7 cells.
**Methods:** To reveal anti proliferative effects of vanilla leaf extract, MTT assay, cell cycle analysis and DNA fragmentation assay was performed as per standard protocols.
**Results:** MTT assay showed decrease in cell viability with increase of dose of extract and revealed IC50 value at 31.2µg/ml. DNA fragmentation was seen in extract treated cells.
**Conclusions:** The results of the present study confirm the antiproliferative property of vanilla leaf extract in MCF-7 cells. This study results conclude vanilla leaf extract as an effective plant source medicament for treating breast cancer.

**Keywords:** Breast cancer, DNA fragmentation, IC50, MTT, Phytochemicals

## INTRODUCTION

Breast cancer is the most commonly diagnosed disease in females and also a major public health problem which needs research to provide a specific treatment.[1] Epidemiological studies revealed that breast cancer had a mortality incidence of rural to urban is 66/8 in India.[2] Taking adverse events of chemotherapy in to consideration, natural products are being explored for anti cancerous effects in the recent researches. MCF-7 cells were widely used for screening anti cancer drugs against breast cancer.[3] *Vanilla planifolia* (vanilla)is a well-known plant which is extensively used as flavouring agent in various food and medicinal products. Because of growing interest for natural products or phytochemicals and their medicinal values, recent studies had shown many medicinal properties of *V. planifolia*. The flavouring agent was obtained majorly from beans of vanilla. This included vanillin, vanillic acid which comprised 250 compounds altogether.[4,5]

Another study revealed that out of 200 compounds, 26 compounds were at a concentration of 1mg.[6-8] Vanilla was first discovered by Aztecs who were Mexicans during 1300's. Aztecs were the first to use it as flavouring agent in drinks. This crop was native to Mexico, due to absence of natural pollinator outside Mexico as stated by the

botanist Charles Morren and this led to the discovery of vanilla's artificial pollination.[9] With this it has explored to other parts of world. Use of vanilla plant as health food agent had ethnical variation. Aztecs used for its stimulant, carminative and aphrodisiac properties. In Venezuela it is used for treating fever and spasm. Argentinian used it for spasms and sexual dysfunctions. Palauans used this plant for treating dysmenorrhoea, fever and hysteria.[10] Recently Sophie et al, has revealed its protective properties against free radicals in skin.[11] The leaf extracts of vanilla species were revealed to possess compounds that have mosquito larvicidal properties at a dose of 0.1-0.2mg/ml.[12] In this study we used extracts of leaves of vanilla for screening its anti-cancerous property against breast cancer cells, MCF-7 cells. With this back ground, we aimed to hypothesise that vanilla leaf extract can be beneficial in treatment of breast cancer.

## METHODS

MCF-7 cells were bought from National Centre for Cell Sciences, Pune. Vanilla leaves were purchased and authenticated. The leaves were shade dried and powdered. The powdered was further treated with ethanol to obtain ethanolic extract. The procedure followed Sun et al, with slight modifications.[12] Two hundred grams of powdered extract was treated with 500ml of ethyl alcohol (98%) and allowed to stand for 2 days. Then the procedure was repeated twice with the precipitate. The obtained alcohol was freezed and filtered. The obtained solution was lyophilized to produce powdered extract of vanilla leaves.

### *Cytotoxicity assay*

The cytotoxicity assay included 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. Culture media specific to this assay was prepared as suggested by previous literature.[13,14] MCF-7 cells were treated with MTT and sample with a dose concentration of 1000µg/ml to 7.8µg/ml dilution. This was compared with control cells. The reaction absorbance was observed by spectrophotometer at 570nm. The cell viability was calculated as per standard formula.[14]

### *DNA fragmentation assay*

The assay method was performed as described by Wyllie et al.[15] The procedure of the assay was depicted in Figure 1. UV transilluminator (Uvitec, England) was used for analysing the DNA fragmentation.

### *Flowcytometry analysis*

MCF-7 cells taken in a Petri dish of 6cm (105 cells) and incubated overnight. Later the medium was changed and DMSO was added to the cells, then incubated for 24 hours. The cells and media were centrifuged at 200g for 7 min and 4ºC. Ice cold phosphate buffered saline containing EDTA(5mM) was used to wash the cells twice. Then the cells were re-suspended in PBS/EDTA (100ml) and fixed with 70% 1ml ethanol. 50ml PI was added after incubating the cells with 50ml RN'ase. The cells were analysed with flow cytometer as per manufacturer's protocol.

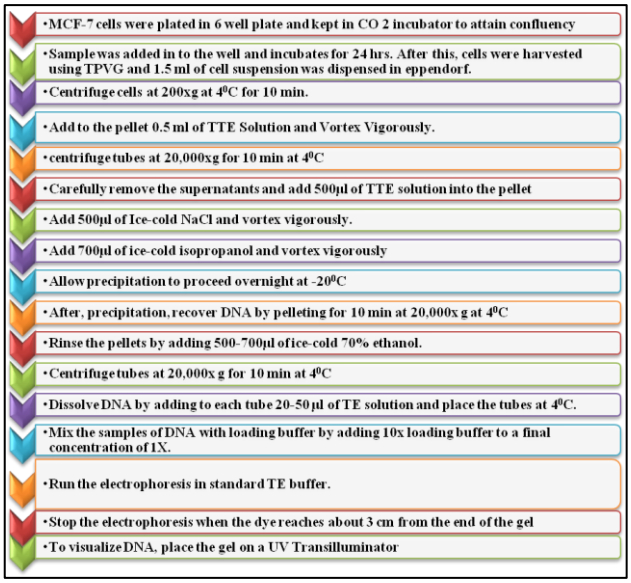



**Figure 1: DNA analysis by gel electrophoresis method.**

## RESULTS

### *MTT assay*

The leaf extract showed a decrease in cell viability with increase in concentration of the extract in a dose dependent manner (Figure 2).

The half maximal inhibitory concentration (IC50) revealed by MTT assay was 31.2µg/ml at a dilution of 1:16. The MCF-7 cell viabilty with various concentrations of vanilla leaf extract was shown the Table 1 below.

It cell viability ranged between 13.32% and 62.42% at an extract concentrations ranging between 1000 µg/ml and 7.8 µg/ml.

**Table 1: Anticancer effect of Vanilla leaf on MCF 7 cells.**

| Concentration (µg/ml) | Dilutions | Absorbance (O.D) | Cell-viability (%) |
|---|---|---|---|
| 1000 | Neat | 0.201 | 13.32 |
| 500 | 1:1 | 0.308 | 20.41 |
| 250 | 1:2 | 0.415 | 27.50 |
| 125 | 1:4 | 0.523 | 34.65 |
| 62.5 | 1:8 | 0.631 | 41.81 |
| 31.2 | 1:16 | 0.729 | 48.31 |
| 15.6 | 1:32 | 0.833 | 55.20 |
| 7.8 | 1:64 | 0.942 | 62.42 |
| Cellcontrol | - | 1.509 | 100 |

***Apoptosis induction by vanilla leaf extract***

The DNA fragmentation was revealed in this assay. The vanilla leaf extract treated MCF-7 cells showed fragmented DNA in comparison to control, depicted in Figure 3. This confirms the antiproliferative effects of the vanilla leaf extract.

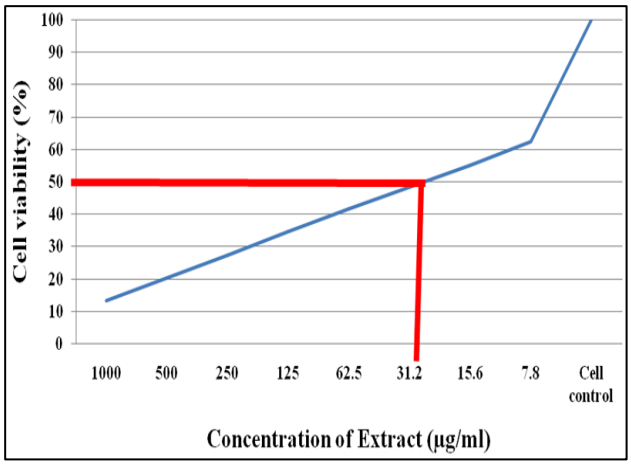
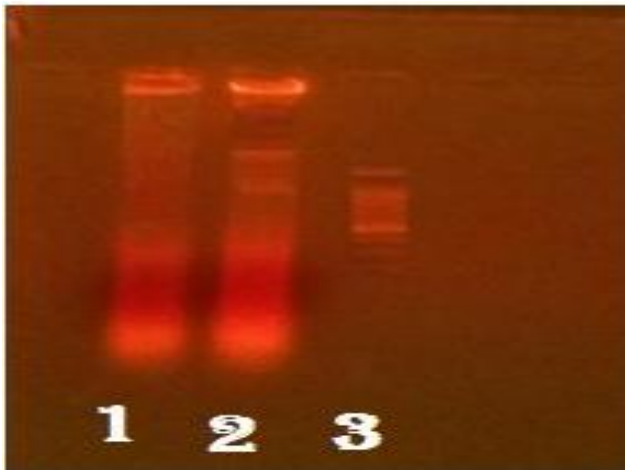



**Figure 2: Cell viability assay of MCF-7 cells.**

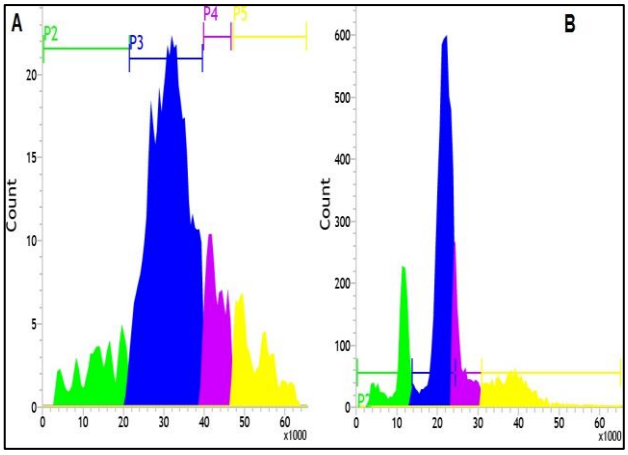



Gel electrophoresis of extracted DNA. 1-indicates the control lane, 2-indicates the vanilla leaf extract treated lane, 3-indicates the marker.

**Figure 3: Gel electrophoresis method for DNA analysis.**

***Effects of Vanilla leaf extract on MCF-7 cell cycle***

Control MCF-7 cells showed 97.6% gated cells. Dead cells were found to be 10%. Majority (64%) of the cells were found to be in G0/G1 stage. 13% of the cells were in S phase and 11% were in G2/M phase. In vanilla leaf extract treated MCF-7 cells, 16% cells showed apoptosis. It was higher than the control. G0/G1 stage showed 59.72%.14% of the cells were in S phase and G2/M phase (Figure 4). Sub G1 population of cells is considered more for the analysis of viable cells and apoptosis.




A- control MCF-7 cells, B- extract treated MCF-7 cells, P2-, P3-, P4-, P5-.

**Figure 4: Cell cycle analysis of control and extract treated MCF-7 cells.**

## DISCUSSION

MCF-7 is a widely used cell line in breast cancer research since years and proved to be a perfect in-vitro model for screening drugs for treating breast cancer or investigating the breast cancer pathology.[16,17] This cell line has provided vast data compared to other breast cancer cell lines.[18] These cells are non-invasive, poorly aggressive and have low metastatic potential.[19,20] Vanilla plant was known for many medicinal properties but its anti-cancerous properties were little known till date. Many phytochemicals were identified in this plant that possess various biological activities. These phytochemicals were used for treating many ailments since decades. It was shown to possess DNA-dependent protein kinase (DNA-PK) inhibitory action which may be responsible for anticancerous effects.[21] A study by Ho K et al, has revealed the anticancerous property of vanilla extract which attained G0/G1 arrest at a dose of 200 microg/ml and G2/M arrest at a dose of 1000 microg/ml. This study concluded that vanilla extract induced apoptosis.[22] In a study, the effect of vanilla extract was observed on growth and metastasis of BALB/c mice 4T1 mammary adenocarcinoma. Orally administered vanilla extract significantly reduced lung metastasized colonies. Vanilla extract inhibited matrix metallopeptidase 9 (MMP-9) secreted by cancer cells which decreased invasion and migration of cancer cells in an in-vitro study. This study concluded vanilla extract to have anti-metastatic activity.[23] Anti-mutagenic property of vanilla extract was revealed for first time by King et al. It induced DNA damage which elicits recombinational DNA repair and further reduced spontaneous mutation.[24] Keshava et al, showed the protective effects of vanilla extract on radiation induced chromosomal damage in V79 cells and indicated anticlastogenic property of vanilla extract.[25] Ohta et al, study investigated vanilla extract on recombination frequency with two plasmid DNA suggested the antimutagenic property of vanilla extract. The frequency of plasmid recombination was significantly higher in presence of vanilla extract. They found that this effect was

due to enhancement of rec A-dependent, error free, pathway of post replication.[26] The anti-proliferative properties of the vanilla leaf extract was shown in this study using cytotoxicity assay and gel electrophoresis DNA extraction. MTT assay showed effective anti-cancerous activity of extract against MCF-7 cells. The assay detects the reduction of MTT salt to blue formazan product by mitochondrial dehydrogenase, which indicates the cell viability.[27] The cell viability of MCF-7 cells decreased with increase of extract dose confirming the anti-cancerous property of the extract with IC50 value at 31.2µg/ml. Several naturally derived alkaloids with prospective anticancer properties against MCF-7 cells, such as berberine, evodiamine, and piperine, have already been reported by other authors.[28] And the reported IC50 values of above mentioned alkaloid compounds suggest that vanilla extract exhibits considerable inhibition than above mentioned alkaloids in MCF-7 cells.[29-31] Graidist et al, revealed IC50 of an extract against MCF-7 cells to be 22.31±0.83µg/mL.[32] E.guineensis extract IC50 value determined by MTT assay was 15.00µg/mL against MCF-7 breast cancer cells.[33] Presence of DNA fragmentation showed by gel electrophoresis method confirms the anti-proliferative effects and apoptosis induction of vanilla leaf extract. DNA fragmentation is an important sign of apoptosis revealing the inhibition of DNA replication and internucleosomal cleavage.[34,35] Anticancer drugs that cause apoptosis selectively to cancer cells, with less side effects to normal cells, are considered important for therapeutic purposes.[36,37] In summary, we suggest that vanilla leaf extract demonstrates selective inhibition of MCF-7 breast cancer cells through inhibition of apoptosis. Furthermore, vanilla leaf extract caused up-regulation DNA fragmentation in MCF-7, which suggests activation of apoptosis. These findings will help to give a basic understanding on the antiproliferative action of vanilla leaf extract in future studies.

## CONCLUSION

The results of the present study conclude that vanilla leaf extract induces cytotoxicity and apoptosis induced cell death in MCF-7 breast cancer cells. Vanilla leaf extract might contain the leading molecule which may be developed as chemotherapeutic agent for treating breast cancer of estrogen receptor positive type. Further research is required to characterize and understand the molecular aspects of the anticancerous effects of the extract.

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