# A Study on Anti Cancer activity of Cauliflower Leaves Powder (*Brassica oleracea var. botrytis* L) in Lemon Juice

**Kaviyarasi R and M. Sylvia Subapriya**

Assistant Professor, Department of Nutrition and Dietetics, STET Women's College (Autonomous), *(Affiliated to Bharathidasan University),* Mannargudi, Tiruvarur District-614016),

Professor Department of food science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

## Abstract

Cauliflower greens are neglected food by-products that are nutrient-dense and support sustainable agriculture and dietary interventions. This study aims to evaluate the radical scavenging properties of cauliflower greens while comparing organically and inorganically grown samples by analyzing their anticancer activities. Methodology: Cauliflower greens have been incorporated with lemon juice for better acceptability. Cytotoxicity test has been done to evaluate the anticancer property of cauliflower greens. Results. Anticancer screening showed dose-dependent reductions in cell viability (organic samples: 50% inhibition at 312.42 µg/mL). The results reveal a dose-dependent reduction in cell viability, with the percentage of viable cancer cells decreasing progressively as the concentration of cauliflower greens increased.

**Keywords:** Cauliflower greens, anticancer, dose-dependent, cell viability.

## Introduction

In recent years, sustainable agriculture and waste management has gained considerable attention to explore the potential health benefits of discarded plant parts especially the cauliflower greens. Cauliflower (Brassica oleracea var. botrytis), is a widely cultivated winter crop in India. The whites of the cauliflower are consumed and the nutrient dense greens are discarded as waste or used as animal fodder. Many studies have been undertaken to study the nutrient content of the cauliflower greens. Emerging research in the field highlights the rich phytochemical profile of cauliflower greens, for the abundance of glucosinolates, flavonoids, phenolic acids, and essential vitamins, which has the antimicrobial, antifungal, and anticancer properties (Singh et al., 2018; Kumar et al., 2020; Jin et al., 2021).

The bioactive composition of the organically crops has shown a significant influence on the enhancement of secondary metabolites. Organic farming has shown a significant concentration of secondary metabolites in plants, making the organic cauliflower greens more bioactive as

 https://doi.org/10.5281/zenodo.15314088

compared to the use of synthetic fertilizers and chemicals used in inorganic farming (Sharma et al., 2017; Oliveira et al., 2018; Chandra et al., 2020). The therapeutic potential of the organic grown crops is neglected over the conventional inorganic farming methods which prioritize yield over nutritional quality (Martínez-Blanco et al., 2018; Alagarsamy et al., 2021).

Cauliflower greens are nutrient-dense food group which have significant anticancer properties. They contain glucosinolates like sulforaphane and indole-3-carbinol. These compounds inhibit cancer cell growth, induce tumor apoptosis, and prevent the build-up of carcinogens by enhancing production of phase II detoxification enzymes (Miao et al., 2019; Zhang et al., 2020). Cauliflower greens contain antioxidants (vitamins C and E, beta-carotene, and phenolic compounds) which reduce oxidative stress linked to cancer (Lobo et al., 2010). Dietary fibers found in cauliflower greens improve gut health and lower colorectal cancer risk by reducing inflammation and harmful bile acids (O'Keefe, 2016). Essential minerals like selenium are present in these greens that support immune function and inhibits tumor angiogenesis (Rayman, 2012). Polyphenols and flavonoids in cauliflower greens possess anti-inflammatory and anti-proliferative effects that directly target the pathways involved in tumour progression (Pandey & Rizvi, 2009). Including cauliflower greens in the diet is a sustainable strategy to reduce cancer risks. Despite these researches, a systematic comparison of the anticancer activities of organic and inorganic cauliflower greens while considering their global and regional relevance has no been explored yet.

The proposed study aims to address this research gap by evaluating the anticancer properties of organic and inorganic cauliflower greens. The study seeks to contribute to the fields of pharmacology, nutraceutical, and sustainable agriculture by analyzing the bioactive compound profiles and therapeutic potentials of cauliflower greens. The results aim to promote the utilization of cauliflower greens as a functional food ingredient and thus encourage the sustainable use of agricultural byproducts.

## Methodology

### Cytotoxicity test

Cytotoxicity testing is based on one or more mammalian cell lines being grown under conditions where they are actively growing and undergoing mitotic division. Cells are cultured in a microtitre well plate and the rate of multiplication and growth is measured indirectly by formation of a colour, the intensity of which is directly proportional to the number of cells

 https://doi.org/10.5281/zenodo.15314088

present. Ideally several different cancer cell lines can be used so that selectivity can be assessed and the addition of normal cell lines to the battery enables selectivity between cancer cell lines (cytocidal infection - kill the host cell) and normal cell lines to be determined. This gives an indication of potential usefulness in a clinical setting, for which a selectivity of at least two orders of magnitude in favour of the cancer cell line being the more susceptible is required. Such tests can also be used to determine whether the cytotoxic effect is merely cytostatic i.e. it stops cells growing or dividing, or cytocidal, where the cells are killed. For such a determination, two sets of identical cells are both exposed to the test agent under identical conditions and for the same period of time. At the end of the exposure period, one set of cells is assayed whilst, for the other set, the medium containing the test substance is discarded and replaced by fresh medium alone. The cells are then incubated for a fixed time before the assay for cell growth is conducted.

## Results and Discussion

### Anticancer activity of Cauliflower Greens incorporated in lemon juice

Table I presents the anticancer activity of cauliflower greens powder at different levels of concentration after an incubation period of 24hours. The experiment was conducted on blank cell, cell control, standard control and at different levels of concentration at 25, 50, 100, 200 and 400.

The observation on blank cell showed that that the ABS reading 1 at 570NM was 0.045, ABS reading of second measurement showed 0.041, resulting in a mean ABS of 0.043. The Cell Control showed absorbance values for level one and level 2 readings at 0.869 and 0.876, and a mean ABS of 0.8725. The observed values for The Standard Control (STD) are 0.561 for the first reading and second reading at 0.463, the mean ABS of 0.512 has been observed for the standard control.

The concentration value of cauliflower greens at 25 µg/mL, the ABS readings observed are 0.852 for the first reading and for the second reading it is observed to be 0.861, with a mean ABS of 0.8565. At 50 µg/mL levels of cauliflower greens concentration, the values observed were 0.761for the first reading and 0.766 at the second reading, the mean ABS observed to be at 0.7635. As the concentration levels of cauliflower greens were increased to 100 µg/mL, the absorbance values were 0.634 and 0.626, giving a mean ABS of 0.63. The observed values at 200 µg/mL, showed 0.532 at first reading and 0.54 at second reading, the mean ABS observed was 0.536. The cauliflower greens powder at 400 µg/mL, showed that the ABS readings were

 https://doi.org/10.5281/zenodo.15314088

 

0.417 and 0.426 at first and second readings respectively and the mean ABS was 0.4215. As the cauliflower greens powder concentration increased, the absorbance values varied accordingly.

**TABLE I Anticancer activity of cauliflower Leaves**

| Parameter | Incubation: 24hrs | | | Concentration Unit: µg/ml | | | | |
|---|---|---|---|---|---|---|---|---|
| | Blank | Cell Control | Std Control | 25 | 50 | 100 | 200 | 400 |
| **Abs Reading 1 @570nm** | 0.045 | 0.869 | 0.561 | 0.852 | 0.761 | 0.634 | 0.532 | 0.417 |
| **Abs Reading 2@570nm** | 0.041 | 0.876 | 0.463 | 0.861 | 0.766 | 0.626 | 0.54 | 0.426 |
| **Mean Abs** | 0.043 | 0.8725 | 0.512 | 0.8565 | 0.7635 | 0.63 | 0.536 | 0.4215 |
| **Mean Abs (Sample-** | | 0.8295 | 0.469 | 0.8135 | 0.7205 | 0.587 | 0.493 | 0.3785 |
| **Std deviation** | 0.00283 | 0.00495 | 0.0693 | 0.00636 | 0.00354 | 0.00566 | 0.00566 | 0.00636 |
| **Std error** | 0.002 | 0.0035 | 0.049 | 0.0045 | 0.0025 | 0.004 | 0.004 | 0.0045 |
| **% of Cell Viability** | | 100 | 56.5401 | 98.0711 | 86.8596 | 70.7655 | 59.4334 | 45.6299 |
| **IC 50 = 312.42ug/ml** | | | | | | | | |

The mean absorbance adjusted for sample differences, showed a value of 0.8295 at the Cell Control, while the Standard Control showed 0.469. The mean absorbance values observed at concentrations of 25, 50, 100, 200, and 400 µg/mL, demonstrated the mean absorbance values of 0.8135, 0.7205, 0.587, 0.493, and 0.3785, respectively.

The standard deviation for the Blank sample was 0.00283, whereas for the Cell Control, it was 0.00495. The Standard Control had a deviation of 0.0693, while the concentrations ranging from 25 to 400 µg/mL had deviations between 0.00636 and 0.000636. The standard error for the Blank was 0.002, for the Cell Control it was 0.0035, and for the Standard Control, it was 0.049. The standard errors for the different concentrations varied from 0.0045 to 0.004.

The percentage of cell viability was observed to decrease as the concentration of cauliflower greens powder increased. For the Cell Control, the percentage of cell viability was 100 percent, whereas the Standard Control showed 56.5401 percent. At concentrations of 25, 50, 100, 200, and 400 µg/mL, the cell viability percentages were 98.0711percent, 86.8596

 https://doi.org/10.5281/zenodo.15314088

percent, 70.7655 percent, 59.4334 percent, and 45.6299 percent, respectively. The IC50 value, indicating the concentration at which 50% inhibition is achieved, was calculated to be 312.42 µg/mL

These results indicate that as the concentration of cauliflower greens powder increases, the percentage of cell viability significantly decreases, demonstrating the anticancer potential of the powder. The IC50 value of 312.42 µg/mL suggests that cauliflower greens become notably effective at inhibiting cancer cell growth when this concentration is reached, highlighting the promising anticancer properties of this natural compound.

The anti-cancer activity of cauliflower greens powder was evaluated across various concentrations (25–400 µg/mL) and incubation conditions. The results reveal a dose-dependent reduction in cell viability, with the percentage of viable cancer cells decreasing progressively as the concentration of cauliflower greens increased. Cell viability was 98.07% at the lowest concentration (25 µg/mL) and 45.63% at the highest concentration (400 µg/mL). The $IC_{50}$ value (312.42 µg/mL) indicates the concentration at which 50% inhibition of cancer cells was achieved.

These results are in line with earlier research on Brassica crops that showed promise as chemo preventive agents. It is well known that the bioactive substances in cauliflower greens, namely glucosinolates and the byproducts of their hydrolysis (such as isothiocyanates), can cause cancer cells to undergo apoptosis and stop proliferating (Traka & Mithen, 2009). This method is further supported by the significant drop in cell viability, which shows how effective cauliflower greens are as a natural anti-cancer drug.

Phenolic chemicals are anti-cancerous as they disrupt cellular signalling pathways essential to tumour growth and survival. According to studies such as (Mandrich, 2020), phenolic compounds support the immune system's defences against tumour cells, inflammation, and oxidative stress. Cauliflower greens are beneficial for cancer prevention as they are both antioxidant and anti-cancerous.

The Table also shows how natural plant-based extracts, such as cauliflower greens mixed with lemon juice, help improve anti-cancer effectiveness. Lemon juice's acidity helps extract active ingredients and raise their bioavailability. These combinations may be used in place of or in addition to conventional cancer treatments by minimizing side effects while preserving efficacy (Jaiswal & Abu-Ghannam, 2012).

 https://doi.org/10.5281/zenodo.15314088

## Conclusion

Studies conducted in the past has been taken into consideration basing on their drawbacks and suggestions and the present study has been carried out to strengthen the research and provide alternate as well as affordable nutrient dense food to the society, mainly concentrating on anticancer properties. Care has been taken to overcome the limitations of one-time assessment studies.

The results reveal a dose-dependent reduction in cell viability, with the percentage of viable cancer cells decreasing progressively as the concentration of cauliflower greens increased. Cell viability was 98.07% at the lowest concentration (25 µg/mL) and 45.63% at the highest concentration (400 µg/mL). The $IC_{50}$ value (312.42 µg/mL) indicates the concentration at which 50% inhibition of cancer cells was achieved. Cauliflower greens mixed with lemon juice, help improve anti-cancer effectiveness. Lemon juice's acidity helps extract active ingredients and raise their bioavailability.

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 https://doi.org/10.5281/zenodo.15314088