



Research Article

EVALUATION OF ANTIOXIDANT, ANTIPROLIFERATIVE AND CHEMOPROPHYLACTIC EFFECT OF APPLE CIDER VINEGAR AGAINST COLON CANCER CELL LINES

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ABSTRACT

Colon cancer is the most threatening cases in the contemporary world due to sedentary lifestyles, changes in eating habits, genetics and etiological factors. The latest statistics proves that it is expected to cause about 50,630 deaths every year. Apple Cider Vinegar (ACV) has been proved to be a very constructive and effective therapy for cancer, hyperlipidemia, hyperglycemia, urolithiasis and skin infections. Objective: The aim of the present work is mainly to evaluate the antioxidant and anticancer properties of apple cider, since there is not much literature supporting the same. Methodology: This work is a laboratory controlled prospective study conducted in our premises. ACV was prepared with ethanol and aqueous fermentation extraction method. Various established (5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml and 30µg/ml) concentrations were prepared for further studies. The phytochemical analysis was performed and the DPPH assay was done for evaluating the antioxidant potency. MTT assay was performed for the evaluation of anticancer action against colon cancer cell lines (HT29) for the diluted concentrations to achieve the minimum inhibitory concentration. Results: The DPPH radical scavenging activity of ACV was found to be ranging from 46.14% to 86.75% for the various concentration ranges. The IC₅₀ value of the extract was found to be 8µg/ml. The HT-29 cancer cell line subjected to various concentrations of apple cider vinegar, resulted in cell viability of 48.90% at 62.5µg/ml. From the graph IC₅₀ value was calculated to be around 50-60µg/ml. Conclusion: It was concluded ACV is biologically active and effective against colon cancer.

Keywords: Apple Cider Vinegar, HT29, IC₅₀, DPPH, MTT

INTRODUCTION

Colon cancer is the second most prevalent cause of cancer deaths in men and women after lung cancer¹. The conversion of precancerous polyps (benign) found in the inner lining of the colon the main etiological reason for colon cancer². Dietary modification, sedentary lifestyles, hereditary and genetic modifications are some of the etiological factors of colon cancer. Refinement and improvising diet can surely control the severity of certain types of digestive tract cancer, especially colon cancer³. Recent research work has shown that almost 75% of all sporadic cases of colorectal cancer are directly affected by dietary intake⁴. Thus, dietary modification is probably one of the best ways for alleviating the risk of developing colon cancer⁵. Cytochrome P450 1A1 metabolizing enzyme is one of the most prominent enzymes responsible for activating the chemical carcinogens in the colon⁶.

People all around the world rely and truly depend on phytomedicine for various ailments which has been reported by WHO, and 33% of the drugs used are from the plant sources⁷. What is apple cider vinegar? ACV is healthy vinegar from the fruits of *Malus domestica*. It contains potent vitamins and minerals such as acetic acid, malic acid, polyphenols, pectin, vitamin C, vitamin B1, B2 and B6, biotin, folic acid, niacin,

pantothenic acid, and a small number of minerals like potassium, sodium, phosphorus, calcium and iron.⁸

The use of ACV is to promote good health and it has a long history in folklore and traditional medicines⁹. There is insufficient evidence from modern, high-quality clinical research to support its health claims¹⁰. Preliminary research is being conducted to impel possible effects on blood glucose level, satiety, anti-infective properties, hypertension and cancer, but no significant clinical studies have supported its use for these condition as of 2017¹¹.

Only basic research has been carried out on Apple Cider Vinegar on anti-diabetic and hypolipidemic profile¹². Hence, further studies on this plant are called for evaluating the pharmacological remedy towards various complicated and chronic disorders¹³. The aim of the present work is mainly to evaluate the antioxidant and anticancer properties of apple cider against colon cancer cell lines HT29, since there is not much literature supporting the same.¹⁴

MATERIALS AND METHODS

Malus domestica, sugar, distilled water, ethanol and blender were taken. Fresh apples were washed and weighed individually, were cut into small pieces (seeds removed) to obtain homogeneous

samples¹⁵. The fruit pulp was homogenised separately in a blender, and the flesh weighed around 1kg¹⁶. Two tablespoons of sugar, along with 70% of ethanol, 30% of water was added to the above to ferment the pulp and was left for a week¹⁷. The supernatant was filtered with the help of Whatman filter paper, and the filtrate was collected, and various test carried out.¹⁸

Phytochemical screening

The supernatant sample was subjected to many phytochemical screenings such as test for alkaloid, carbohydrates, proteins, steroids, sterols, phenols, tannins, flavonoids¹⁹.

DPPH assay

The free radical scavenging activity of Apple Cider vinegar was measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Hence 0.1 mM solution of DPPH in ethanol was prepared. This DPPH solution (1 ml) was added to 3 ml of our product in ethanol at different concentration (5, 10, 15, 20, 25, 30 µg/ml), and the mixture was shaken vigorously and concede to stand at room temperature for 30 minutes, the absorbance was then measured at 517 nm by using spectrophotometer. Ascorbic acid was used as a source compound and experiment was done in triplicate. The IC₅₀ value is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using the log dose inhibition curve. The lower absorbance of the reaction mixture indicates high free radical activity. The percent DPPH scavenging effect was calculated by using the following equation:

$$\text{DPPH scavenging effect (\%)} \text{ or Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A₀ was the Absorbance of control reaction and A₁ was the Absorbance in the presence of test or standard sample²⁰.

MTT assay

Cell lines were procured from the National Centre for Cell Sciences, Pune (NCCS). Cells (1 × 10⁵/well) were plated in 24-well plates and incubated in 37°C with 5% CO₂ condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4). 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed, and the concentration required for a 50% inhibition (IC₅₀) was determined graphically. The % cell viability was calculated using the following formula:

$$\% \text{ Cell viability} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100$$

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments²¹.

Table 1: Phytochemical Report of Apple Cider Vinegar

S. No	Phytochemical Test	Ethanolic
1	Test for Alkaloids	-
2	Test for Steroids	-
3	Test for Phenols	+
4	Test for Tannins	+
5	Test for Flavonoids	+
6	Test for Saponins	-
7	Test for Fixed oils	-
8	Test for Gums and Mucilage	-
9	Test for Glycosides	-
10	Test for Triterpenoids	-
11	Test for Carbohydrates	+

Table 2: Antioxidant Assay of Apple Cider Vinegar using DPPH Assay

Concentration (µg/ml)	Percentage inhibition of test (%)	Percentage inhibition of standard (%)
5	46.14±0.8	52.03±5.5
10	52.87±1.9	64.98±3.2
15	63.12±2.3	71.09±1.1
20	70.98±1.7	77.44±2.9
25	77.81±1.3	82.56±10.4
30	86.75±5.9	87.35±2.5

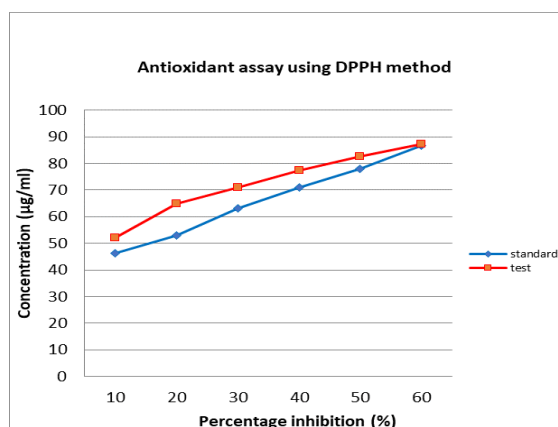


Figure 1: DPPH Assay of Apple Cider Vinegar

Table 3: MTT assay of apple cider vinegar against HT-29 cell lines

S. No	Concentration (µg/ml)	Dilutions	Absorbance(O.D)	Cell viability (%)
1	1000	Neat	0.079	11.53
2	500	1:1	0.156	22.77
3	250	1:2	0.202	29.48
4	125	1:4	0.278	40.58
5	62.5	1:8	0.335	48.90
6	31.2	1:16	0.409	59.70
7	15.6	1:32	0.488	71.21
8	7.8	1:64	0.574	83.79
9	Cell control	-	0.684	100

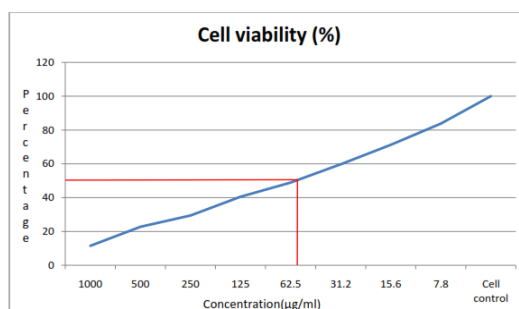
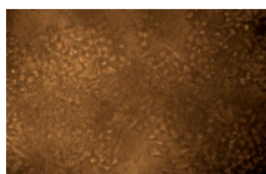


Figure 2: MTT Assay of Apple Cider Vinegar against HT-29 Cell Lines

Anticancer effect of Sample on HT 29 cell line

Normal HT29 cell line



Toxicity-1000 µg/ml



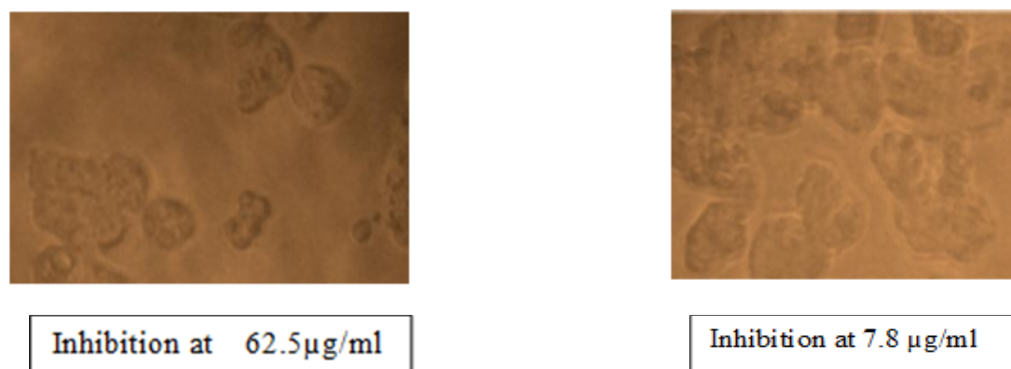


Figure 3: MTT Assay of Apple Cider Vinegar against HT-29 Cell Lines

RESULTS

ACV contains potential anticancer properties, and the phytochemical analysis revealed the presence of carbohydrates, polyphenols, proteins and flavonoids and the data were tabulated in Table 1.

DPPH ASSAY

The DPPH radical scavenging capacity of Apple Cider Vinegar and ascorbic acid were shown in figure 1. The percentages of DPPH radical scavenging activity of Cider were found to be 46.14, 52.87, 63.12, 70.98, 77.81 and 86.75 % at the concentrations of 5, 10, 15, 20, 25 and 30 µg/ml ($P < 0.05$) respectively. The highest percentage of inhibition was exhibited 86.75 % ($P < 0.05$) at the highest concentration. The IC_{50} value of the extract was found to be around 8µg/ml. The results of DPPH assay have been tabulated in table 2 and figure 1.

MTT ASSAY

The MTT screening of Apple Cider Vinegar resulted from potent anticancer activity against HT-29 (colon cancer) cell lines. The percentage of cancer cell inhibition profile was found to be concentration dependent. The maximum concentration used in the study was 1000µg/ml. The inhibitory property of apple cider vinegar are tabulated in table 3 and figure 2 and figure 3.

The HT-29 cancer cell line, when subjected to various concentrations of apple cider vinegar, resulted in cell viability of 48.90% at 62.5µg/ml. From the graph IC_{50} value was calculated to be around 50-60µg/ml.

DISCUSSION

Although various therapeutic options are available in the therapy of colon cancer, the response had side effects too which was not appreciable. Our study has proven the chemo preventive effect of ACV against HT29 cell lines with minimal side effects with good to excellent results as stated priorly. The phytochemical analysis has revealed that apple cider vinegar has high phenols, proteins and flavonoids which could be one of the best and natural treatment for colon cancer. With surgical and radiation therapy advancements treating for colon cancer, this studies using ACV was considered better than surgical procedures and also with a privilege of absence of side effects. Consuming a mild dose of ACV everyday can certainly show improving results.

CONCLUSION

The present work aimed at the evaluation of the chemo protective effect of Apple cider vinegar by DPPH assay and MTT assay methods for antioxidant and anti-proliferative mechanisms. It also suggested that Apple Cider Vinegar could be a potential source of a natural antioxidant drink and thus be useful as a therapeutic agent against cancer.

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