ANTICANCER EFFICACY AND PHYTOCHEMICAL STUDY OF 50% ETHANOLIC EXTRACT OF ACMELLA CILIATA: AN IN VITRO APPROACH

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ABSTRACT

Antioxidants are compounds that inhibit oxidation, a chemical reaction that can produce free radicals and chain reactions that may damage the cells of organisms, free radicals play a part in cancer heart disease, stroke and other diseases of ageing. The anticancer activity can be evaluated by using in vitro MTT assay method. An in-vitro antioxidant study was performed on collected, authenticated and extracted 50% ethanolic extract of *Acmella ciliata*. The methods for assessing antioxidant activity were the DPPH radical scavenging assay, hydrogen peroxide radical scavenging assay, and FRAP assay. The results obtained in the present study indicated that 50% ethanolic extract of *Acmella ciliata* has appreciable antioxidant capacity. Hence, these can be used as natural sources of antioxidants as they could have great importance as therapeutic agents in slowing or preventing the progress of ageing and oxidative enhancement related cancer treatment.

Keywords: Antioxidant; *Acmella ciliata*; Free radicals, DPPH Hydrogen peroxide; scavenging cancer.

INTRODUCTION

Agents are being used for the treatment of cancer. Several anticancer agents from plants include; taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from epipodo phyllotoxin are in clinical use all over the world. Numerous cancer research studies have been conducted using traditional medicinal plants to discover new therapeutic agents that lack the toxic side effects associated with current chemotherapeutic agents and the drugs under clinical phytomedicines have increased dramatically in the last two decades. It has been also reported that more than 50% of all modern drugs in clinical use are natural products, many of which have been recognized to have the ability to include apoptosis in various cancer cells of human originals, there is an urgent need to develop many effective and less toxic drugs. In vitro studies. Geinstien in plants such as parsley and soy foods inhibits protein try sine kinase, thereby disrupting signal transduction and inducing cell differentiation. The oral route is the most suitable and preferable route for drug administration to reach systemic circulation due to its low cost and easy administration. However, the success of the conventional dosage form is limited due to its residence time. Hence mucoadhesive microsphere drug delivery systems are used to prolong the residence time at the site of application, maintain therapeutically effective plasma drug concentration levels for a longer duration, reduce the dosing frequency and minimize fluctuations in the plasma drug concentration at the steady state in a controlled and reproducible manner Cancer cell lines have been widely used for research purposes and proved to be a useful tool in the genetic approach, and their characterization shows that they are, in fact, an excellent model for the study of the biological mechanisms involved in cancer. The use of cancer cell lines allowed an increment of the information about the deregulated genes and signalling pathways in this disease. Furthermore, the use of the cell model was in the origin of the development and testing of anticancer drugs presently used and in the development of new therapies, but also as an alternative to transplantable animal tumours in chemotherapeutics testing. The use of the appropriate *in vitro* model in cancer research is crucial for the investigation of genetic, epigenetic and cellular pathways for the study of proliferation deregulation, apoptosis and cancer progression, to define potential molecular markers and for the screening and characterization of cancer therapeutics. The results of the research in cancer cell lines are usually extrapolated to *in vivo* human tumours and its importance as models for drug testing and translational study have been recognized by many biomedical and pharmaceutical companies. The study utilized various cancer cell lines derived from different species and cancer types, each with distinct morphological characteristics. The HeLa cell line, originating from *Homo sapiens*, represents cervix adenocarcinoma with an epithelial morphology. Similarly, the MCF-7 cell line, also from *Homo sapiens*, is associated with breast adenocarcinoma and exhibits epithelial morphology. The U87MG cell line, another *Homo sapiens* derivative, is linked to glioblastoma-astrocytoma with epithelial morphology.In addition, the HT-29 cell line from *Homo sapiens* pertains to colon adenocarcinoma and is epithelial in nature. The A549 cell line, representing lung carcinoma in *Homo sapiens*, also exhibits epithelial morphology. The HEP-G2 cell line, associated with hepatocellular carcinoma, maintains an epithelial morphology as well.For hematological malignancies, the K-562 cell line from *Homo sapiens* is characterized by

chronic myeloid leukemia with lymphoblast morphology. Lastly, the Cos7 cell line, derived from *Homo sapiens*, is associated with SV40-transformed kidney cells and exhibits fibroblast morphology. To conduct an in vitro anticancer assessment of the hydroalcoholic extract of *Acmella ciliata*, the following steps were undertaken: The first step involved the collection of *Acmella ciliata* plant material from a suitable location. The plant was then authenticated by a qualified botanist or plant taxonomist to ensure that the correct species was being used for the study. This step is crucial to verify the identity of the plant, ensuring that the subsequent experiments are conducted with the intended species.After authentication, the plant material was cleaned, dried, and subjected to extraction using a hydroalcoholic solvent (a mixture of water and ethanol). The hydroalcoholic extraction process was carried out to obtain the bioactive compounds present in the plant. This step involved maceration or another appropriate extraction method to ensure the efficient isolation of phytochemicals from the plant material.The prepared hydroalcoholic extract underwent phytochemical screening to identify the presence of various bioactive compounds. This qualitative analysis helped in determining the types of phytochemicals (such as alkaloids, flavonoids, tannins, saponins, etc.) present in the extract. Phytochemical screening provides insight into the potential therapeutic properties of the plant extract and helps in understanding its medicinal value.**:** To assess the antioxidant potential of the hydroalcoholic extract, the following assays were performed: This assay was used to evaluate the ability of the extract to neutralize free radicals, specifically DPPH radicals. The reduction in absorbance indicated the scavenging potential of the extract, providing a measure of its antioxidant activity.This test was conducted to assess the extract's capability to scavenge hydrogen peroxide radicals, which are reactive oxygen species that contribute to oxidative stress in cells.The FRAP assay measured the reducing power of the extract by converting ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The increased absorbance in this assay indicated a higher antioxidant capacity of the extract.The anticancer potential of the hydroalcoholic extract was evaluated through the following assay The MTT assay was employed to assess the cytotoxic effects of the extract on various cancer cell lines. This colorimetric assay measures cell viability by converting the MTT reagent into a formazan product in living cells. The extent of the conversion is directly proportional to the number of viable cells. A decrease in cell viability after treatment with the extract indicated its potential anticancer activity.Each of these steps was meticulously performed to investigate the therapeutic potential of *Acmella ciliata* and its hydroalcoholic extract in cancer treatment.

MATERIALS AND METHODS

Authentication

The plant material used in this study was formally identified and authenticated as *Acmella ciliata* (Kunth) Cassini by Dr. Minoo Divakaran, a distinguished professor in the Department of Botany at Providence Women’s College, Kozhikode. Dr. Divakaran's expertise in botany, particularly in plant taxonomy, ensured the accurate classification of the species. This authentication process is a critical step in the research, as it guarantees that the plant material used for the study is indeed the correct species, eliminating any potential errors that could arise from misidentification. Dr. Divakaran's professional validation provides a solid foundation for the subsequent scientific investigations of the plant's bioactive properties.

Extraction

The extraction process of *Acmella ciliata* whole plant was conducted using the cold maceration technique, a method chosen for its effectiveness in preserving the bioactive compounds present in the plant material. Initially, 1000 grams of dried and finely powdered *Acmella ciliata* plant material was carefully weighed and prepared for extraction. The plant powder was then immersed in 1500 millilitres of ethanol, the chosen solvent for this extraction, ensuring that the plant material was thoroughly washed and saturated with the solvent.

The cold maceration process involved allowing the plant material to remain in contact with the ethanol for a specified period, enabling the solvent to penetrate the plant tissues and dissolve the bioactive compounds. This gentle extraction method helps to prevent the degradation of sensitive compounds that might occur under high temperatures.

After the extraction was completed, the mixture was subjected to filtration to separate the plant residue from the liquid extract. The excess solvent was then evaporated using an appropriate method, such as a rotary evaporator, to concentrate the extract. This step ensured that only the active constituents of the plant were retained, leaving behind a dried extract.

To protect the dried extract from environmental factors such as light, moisture, and air, it was carefully stored in a container covered with aluminum foil. This precautionary measure helps maintain the stability and potency of the extract for future analysis.

Finally, the percentage yield of the extract was calculated to determine the efficiency of the extraction process. The percentage yield was determined using the following formula:

Percentage yield (%) = (Weight of extract / Weight of plant material taken) × 100.

This calculation provided insight into the amount of extractable material obtained from the plant, which is an important parameter in evaluating the success of the extraction process.

Phytochemical Screening

a. Test for Alkaloids

1. **Dragendorff's Test:** To detect the presence of alkaloids in the extract, it was treated with Dragendorff's reagent, which is a solution of potassium bismuth iodide. The formation of an orange-brown precipitate after the addition of the reagent confirmed the presence of alkaloids in the extract.
2. **Hager's Test:** In this test, the extract was treated with Hager's reagent, which is a saturated solution of picric acid. The appearance of a yellow-colored precipitate indicated the presence of alkaloids in the plant extract, further validating the results obtained from the previous test.
3. **Mayer's Test:** The extract was then treated with Mayer's reagent, a solution containing potassium mercuric iodide. The development of a cream-colored precipitate following the addition of the reagent was a positive indication of alkaloids in the extract.
4. **Wagner's Test:** Finally, the extract was subjected to Wagner's test, which involves the use of iodine-potassium iodide solution (Wagner's reagent). The formation of a reddish-brown precipitate upon treatment confirmed the presence of alkaloids.

b. Test for Carbohydrates

1. **Molisch Test:** To test for carbohydrates, the extract was treated with Molisch's reagent, which contains α-naphthol in 95% ethanol. A few drops of concentrated sulfuric acid (H_2SO_4) were then carefully added along the sides of the test tube. The appearance of a violet ring at the junction of the two liquids indicated the presence of carbohydrates in the extract.
2. **Fehling's Test:** A small portion of the extract was mixed with Fehling's reagent, which consists of Fehling's reagent A (copper sulfate in water) and Fehling's reagent B (sodium potassium tartrate). The mixture was then heated in a water bath. The formation of a red precipitate confirmed the presence of reducing sugars in the extract.
3. **Barfoed's Test:** For this test, the extract was treated with Barfoed's reagent, a solution containing copper acetate in water and glacial acetic acid. The mixture was heated in a water bath, and the appearance of a red precipitate indicated the presence of monosaccharides in the extract.
4. **Benedict's Test:** The extract was treated with Benedict's reagent, a mixture of copper sulfate, sodium citrate, and sodium carbonate in water, and then heated for 10 minutes. The formation of a red precipitate signified the presence of reducing sugars in the extract, corroborating the results from Fehling's test.

c. Test for Proteins and Amino Acids

1. **Biuret Test:** To test for proteins, the extract was treated with 10% sodium hydroxide (NaOH) and a few drops of 1% copper sulfate ($CuSO_4$) solution. After mixing, the appearance of a pink to violet color indicated the presence of proteins in the extract.
2. **Millon's Test:** In Millon's test, the extract was treated with 5 mL of Millon's reagent. Upon warming, a white precipitate formed, which then turned brick red, or dissolved to give a red color, confirming the presence of proteins in the extract.

d. Test for Flavonoids

1. **Ferric Chloride Test:** The extract was tested for flavonoids by treating it with a few drops of ferric chloride solution. The formation of a blackish-blue color signified the presence of flavonoids in the extract.
2. **Lead Acetate Test:** In this test, the extract was treated with lead acetate solution. The appearance of a yellow precipitate indicated the presence of flavonoids, supporting the findings from the Ferric Chloride Test.

e. Test for Saponins

1. **Foam Test:** To test for saponins, 0.5 g of the extract was shaken vigorously with 2 mL of water. If the foam produced persisted for 10 minutes, it confirmed the presence of saponins in the extract.

Antioxidant Activity Screening Methods: DPPH Radical Scavenging Assay

The antioxidant activity of the plant extracts was evaluated using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, a widely used method for assessing the free radical scavenging ability of compounds. This assay was performed according to the procedure described by Guchu, B.M., with some modifications to suit the experimental conditions.

Preparation of Samples

To begin, five different concentrations of the plant extracts were prepared, specifically 10 mg/ml, 20 mg/ml, 40 mg/ml, 80 mg/ml, and 100 mg/ml, using ethanol of analytical grade as the solvent. This range of concentrations allowed for the evaluation of the dose-dependent antioxidant activity of the extracts.

Reaction Mixture

The reaction mixture was carefully prepared by combining 1.0 ml of 0.3 mM DPPH solution in ethanol, 1.0 ml of the plant extract at the desired concentration, and 1.0 ml of methanol, resulting in a total volume of 3.0 ml. The DPPH solution acts as a stable free radical, and its reduction by antioxidants in the plant extract leads to a color change, which can be quantitatively measured.

Incubation and Measurement

The prepared reaction mixtures were incubated in the dark for 10 minutes. This step was crucial to prevent any interference from light, which could affect the stability of the DPPH radicals. After the incubation period, the absorbance of each mixture was measured at 517 nm using a colorimeter. This wavelength is specifically selected for its sensitivity to the color change associated with DPPH radical reduction.

Experimental Replication

To ensure the accuracy and reliability of the results, all experiments were performed in triplicate. This repetition minimized experimental errors and provided a more robust data set for analysis.

Calculation of Inhibition Percentage

The percentage of DPPH radical scavenging activity, or inhibition, was calculated using the following formula:

This calculation provided the inhibition percentage, indicating how effectively the plant extract neutralized the DPPH radicals at each concentration.

Determination of IC_{50}

The half-maximal inhibitory concentration (IC_{50}) of the plant extracts was then determined. This value represents the concentration of the extract required to inhibit 50% of the DPPH radicals. It was computed by plotting the percentage inhibition against the extract concentrations and determining the point at which 50% inhibition was achieved.

The DPPH assay provided a clear indication of the antioxidant potential of the plant extracts, allowing for the comparison of their efficacy in scavenging free radicals and potentially contributing to their overall therapeutic benefits.

Inhibition (%) = $(A_0 – A_1/ A_0) \times 100$

Hydrogen Peroxide Radical Scavenging Assay

The hydrogen peroxide radical scavenging assay was conducted following the method outlined by Chanda and Dave, with slight modifications to suit the specific experimental requirements. This assay is designed to measure the ability of plant extracts to neutralize hydrogen peroxide radicals, a common reactive oxygen species (ROS) that can contribute to oxidative stress in biological systems.

Preparation of Hydrogen Peroxide Solution

A 20 mM solution of hydrogen peroxide (H_2O_2) was prepared in phosphate buffer (50 mM, pH 7.4). This buffer provides a stable environment that closely mimics physiological conditions, ensuring accurate measurement of the radical scavenging activity.

Determination of Hydrogen Peroxide Concentration

The concentration of hydrogen peroxide in the solution was determined by measuring its absorbance at 230 nm using a colorimeter. This wavelength was selected for its specificity in detecting hydrogen peroxide, allowing for precise quantification.

Sample Preparation

Five different concentrations of the plant extract were prepared in distilled water, specifically 10 mg/ml, 20 mg/ml, 40 mg/ml, 80 mg/ml, and 100 mg/ml. These varying concentrations were used to evaluate the dose-dependent scavenging activity of the extract.

Reaction Setup

For the scavenging assay, 1 ml of each plant extract concentration was added to 2 ml of the hydrogen peroxide solution prepared in phosphate buffer. The reaction mixture was incubated for 10 minutes, allowing sufficient time for the extract to interact with the hydrogen peroxide radicals.

Measurement of Absorbance

After the 10-minute incubation, the absorbance of the reaction mixture was measured at 230 nm using a colorimeter. The absorbance values obtained were compared to a blank solution, which consisted of phosphate buffer without hydrogen peroxide. This blank served as a reference point, ensuring that any observed changes in absorbance were solely due to the interaction between the extract and hydrogen peroxide.

Calculation of Hydrogen Peroxide Scavenging Activity

The percentage of hydrogen peroxide scavenging by the plant extract was calculated using the following above formula: This calculation provided a clear indication of the plant extract's effectiveness in scavenging hydrogen peroxide radicals, expressed as a percentage of inhibition. The results from this assay contribute to the overall understanding of the plant extract's antioxidant potential, particularly its capacity to neutralize reactive oxygen species, which can play a significant role in mitigating oxidative stress-related damage in biological systems.

Ferric ion reducing antioxidant power (FRAP) assay.

Ferric Reducing Antioxidant Power (FRAP) Assay Procedure

The Ferric Reducing Antioxidant Power (FRAP) assay is a widely used method to assess the antioxidant potential of plant extracts by measuring their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This reduction is indicated by a change in color, which can be quantified by measuring the absorbance of the reaction mixture.

Preparation of FRAP Solution:

To begin the assay, 3.6 mL of the FRAP solution was prepared and added to 0.4 mL of distilled water. This mixture was then incubated at 37°C for 5 minutes. The incubation step is essential as it allows the FRAP solution to stabilize before it interacts with the plant extract.

Reaction with Plant Extract:

Following the incubation, the FRAP solution was mixed with a specific concentration of the plant extract (80 µL). This mixture was then incubated at 37°C for an additional 10 minutes.

The purpose of this step is to provide ample time for the plant extract to react with the FRAP solution, facilitating the reduction of ferric ions to ferrous ions if antioxidant compounds are present.

Measurement of Absorbance:

After the incubation period, the absorbance of the reaction mixture was measured at 593 nm using a spectrophotometer. The absorbance at this wavelength is directly proportional to the concentration of ferrous ions produced, which in turn reflects the antioxidant capacity of the plant extract.

Calibration Curve Construction:

To quantify the antioxidant activity of the plant extract, a calibration curve was constructed using a series of known concentrations of ferrous sulfate ($FeSO_4 \cdot 7H_2O$). Specifically, five concentrations were prepared: 0.1 mM, 0.4 mM, 0.8 mM, 1.0 mM, 1.12 mM, and 1.5 mM. The absorbance of these standard solutions was measured under the same conditions as the sample solutions. The calibration curve, created by plotting absorbance against the concentration of $FeSO_4$, allows for the determination of the antioxidant power of the plant extract by comparing its absorbance to that of the standards. This provides a quantitative measure of the extract's ability to act as a reducing agent, thereby indicating its potential antioxidant efficacy.

The FRAP assay results contribute to a comprehensive evaluation of the plant extract's ability to combat oxidative stress, which is vital in understanding its therapeutic potential.

% Inhibition = (1-AS/AB) X 100

Where,

AS = Absorbance of sample

AB = Absorbance of blank

Cytotoxicity Assay by Direct Microscopic observation

Entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula:% of viability

$$\frac{\text{Mean OD Samples x } 100}{\text{Mean OD of control group}}$$

RESULTS AND DISCUSSION

Extraction Process and Yield Calculation

The extraction process involved obtaining a sticky, resinous extract from the powdered plant material through cold maceration. Cold maceration is a technique where the plant material is soaked in a solvent at room temperature to preserve the bioactive compounds while avoiding the degradation that can occur with heat.

Preparation of Extract:

1. Plant Material: The plant material used for extraction was finely powdered to increase the surface area and improve the efficiency of the extraction process.
2. Extraction Method: The powdered plant material was subjected to cold maceration. In this process, the plant powder was soaked in a suitable solvent at room temperature for an extended period, allowing the active constituents to dissolve into the solvent.

After the completion of the maceration process, a total of 60.39 grams of sticky, resinous extract was obtained.

3. Calculation of Percentage Yield:

To assess the efficiency of the extraction process, the percentage yield of the extract was calculated using the following formula:

- Weight of Plant Material Taken**:** 1 kilogram (or 1000 grams)

Substituting these values into the standard formula gives: 6.039%

This calculation indicates that the extraction process yielded 6.039% of the original plant material weight in the form of the extract. This yield percentage reflects the efficiency of the cold maceration process in isolating the desired compounds from the plant material.

Phytochemical Screening Results

The phytochemical screening of the plant extract involved a series of tests to identify the presence of various bioactive compounds. Here is a detailed account of the results obtained from each test:

Alkaloid Tests:

- Dragendroff’s Test: Positive
- Hager’s Test: Positive
- Mayer’s Test: Positive
- Wagner’s Test: Positive

The positive results for Dragendroff’s, Hager’s, Mayer’s, and Wagner’s tests indicate a significant presence of alkaloids in the plant extract. Alkaloids are a diverse class of naturally occurring organic compounds that typically contain basic nitrogen atoms. They are known for their pharmacological activities, which include analgesic, antimalarial, antibacterial, and anticancer properties. The presence of these alkaloids suggests that the plant may have potential therapeutic applications in treating various diseases.

Carbohydrate Tests:

- Molisch’s Test: Positive
- Fehling’s Test: Positive
- Barfoed’s Test: Positive
- Benedict’s Test: Positive

The positive outcomes of Molisch's, Fehling's, Barfoed's, and Benedict's tests confirm that the plant extract contains carbohydrates. Carbohydrates play a crucial role in plant metabolism and can offer therapeutic benefits, including energy provision, immune system enhancement, and inflammation reduction. The presence of carbohydrates indicates that the plant may be a valuable source of energy and could contribute additional health benefits.

Protein and Amino Acid Tests:

- Biuret Test: Negative
- Millon's Test: Negative

The negative results for both the Biuret and Millon tests suggest that the plant extract does not contain significant amounts of proteins or amino acids. Proteins and amino acids are vital for various physiological processes, such as enzyme activity and tissue repair. Although the plant extract may not be a significant source of these macromolecules, it might still possess other bioactive compounds contributing to its therapeutic properties.

Flavonoid and Tannin Tests:

- Ferric Chloride Test: Positive
- Lead Acetate Test: Positive

The positive results for Ferric Chloride and Lead Acetate tests indicate the presence of flavonoids and tannins in the plant extract. Flavonoids are known for their antioxidant properties and their potential to reduce the risk of chronic diseases like heart disease and cancer. Tannins possess astringent properties and are beneficial in wound healing and inflammation reduction. The presence of these compounds suggests that the plant may have significant antioxidant and anti-inflammatory properties.

Saponin Test:

- Foam Test: Positive

The positive result for the Foam test indicates that the plant extract contains saponins. Saponins are glycosides with detergent-like properties and are recognized for their ability to enhance the immune system, reduce cholesterol levels, and exhibit anticancer effects. The presence of saponins suggests that the plant may be useful in boosting immune function and offering other health benefits.The phytochemical screening of the plant extract reveals a diverse range of bioactive compounds, including alkaloids, carbohydrates, flavonoids, tannins, and saponins. Each of these compounds has potential therapeutic benefits, such as antioxidant, anti-inflammatory, and immune-enhancing properties. These findings highlight the plant's potential as a source of natural health benefits and its applicability in various medicinal uses.

Table.No:1: Phytochemical Screening

Test	**Experiment**	**Result**
Alkaloids	Dragendroff's test	+
	Hager's test	+
	Mayer's test	+
	Wagner's test	+
Carbohydrates	Molisch test	+
	Fehlings test	+
	Barfoed's test	+

	Benedict's test	+
Protein and amino acid	Biuret test	-
	Millon's test	-
Flavanoids and Tanins	Ferric chloride test	+
	Lead acetate test	+
Saponins	Foam test	+

In-Vitro Antioxidant Assays

DPPH Detailed Analysis of Antioxidant Activity at Various Concentrations

The antioxidant activity of the plant extract was assessed at several concentrations: 10 mg/ml, 20 mg/ml, 40 mg/ml, 80 mg/ml, and 100 mg/ml. The following absorbance values and percentage inhibitions were recorded for each concentration:

At 10 mg/ml Concentration:

Recorded Absorbance Values**:** 0.088, 0.086, and 0.087

Average Absorbance: 0.0873

Percentage Inhibition: 2.2471 ± 0.5618 At this concentration, the extract exhibited a modest level of antioxidant activity, as indicated by the relatively low percentage inhibition.

At 20 mg/ml Concentration:

Recorded Absorbance Values: 0.081, 0.083, and 0.080

Average Absorbance: 0.0836

Percentage Inhibition: 8.6141 ± 0.5618The antioxidant activity improved compared to the 10 mg/ml concentration, with a higher percentage inhibition reflecting enhanced scavenging of free radicals.

At 40 mg/ml Concentration:

Recorded Absorbance Values: 0.078, 0.074, and 0.071

Average Absorbance: 0.0743

Percentage Inhibition: 16.4793 ± 7.3033 A further increase in concentration led to a notable increase in antioxidant activity, as shown by the higher percentage inhibition.

At 80 mg/ml Concentration:

Recorded Absorbance Values: 0.064, 0.069, and 0.060

Average Absorbance**:** 0.0643

Percentage Inhibition: 27.7152 ± 2.247

This concentration demonstrated significant antioxidant activity, with a substantial increase in percentage inhibition compared to the lower concentrations.

At 100 mg/ml Concentration:

Recorded Absorbance Values: 0.058, 0.052, and 0.049

Average Absorbance: 0.053

Percentage Inhibition: 40.4494 ± 5.0562

The highest concentration resulted in the greatest antioxidant activity, as evidenced by the highest percentage inhibition recorded. Free radicals are highly reactive molecules that cause oxidative damage to cells by stealing electrons from other molecules in an attempt to stabilize themselves.

While the body's endogenous antioxidant enzyme system typically manages free radicals, excessive free radical production or a failure in the antioxidant defense mechanisms can lead to cellular damage and various diseases. Therefore, the discovery of effective natural antioxidants is crucial for reducing the risk of chronic diseases. The antioxidant activity of the methanol extract of *Passiflora edulis* (P.edulis) was evaluated using several in-vitro models. Due to the diverse nature of free radicals, it is essential to test the extract against multiple types of free radicals to comprehensively assess its antioxidant potential. The DPPH radical scavenging assay, which measures the reduction in absorbance at 519 nm, was used to evaluate the capacity of the extract to neutralize DPPH radicals. The results indicate that the antioxidant activity of the plant extract increases with higher concentrations. The varying degree of DPPH radical scavenging activity observed with different doses underscores the effectiveness of the extract in reducing free radical concentrations and suggests its potential as a potent antioxidant. The DPPH scavenging assay results indicate that ascorbic acid (standard) exhibits stronger antioxidant activity across all concentrations compared to the plant extract. At 100 mg/mL, ascorbic acid achieved a higher percentage inhibition (50.4494%) compared to the plant extract (40.4494%). However, the plant extract demonstrated moderate antioxidant activity, with an IC50 value of 107 mg/mL, which is slightly lower than that of ascorbic acid (112 mg/mL). This suggests that the plant extract may have a comparable antioxidant potential but is less effective than ascorbic acid at lower concentrations.

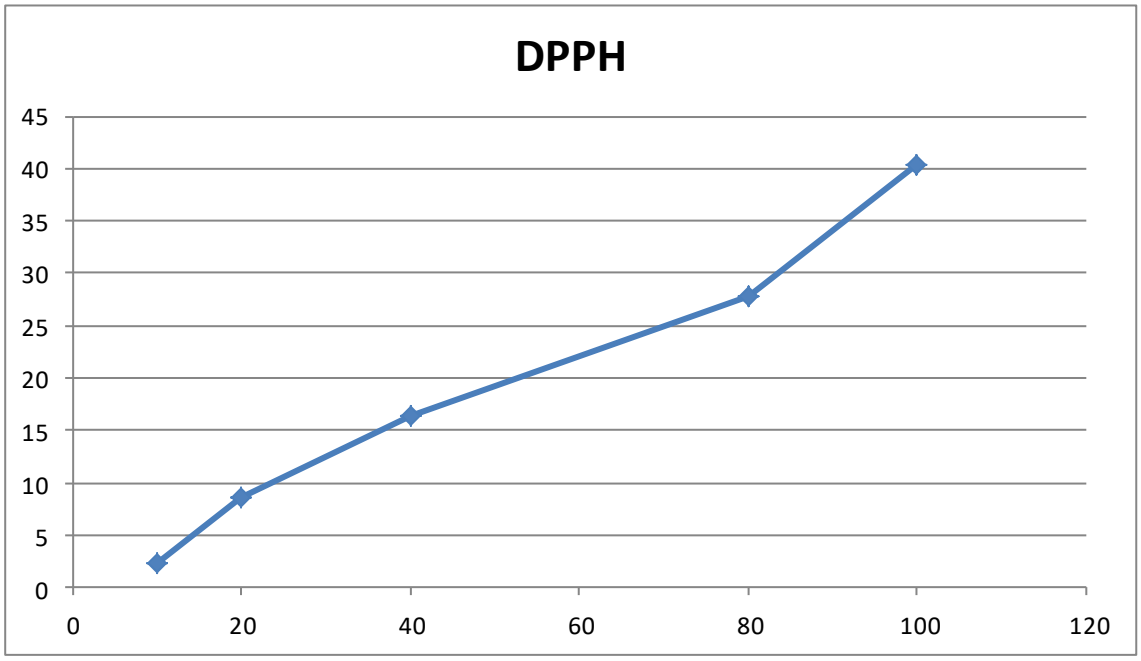
Table No.2: DPPH Scavenging Assay of Extract

Concentration (mg/ml)	**Absorbance**				**% Inhibition**
	A1	**A2**	**A3**	**Average**	
10	0.088	0.086	0.087	0.0873	2.2471±0.5618
20	0.081	0.083	0.080	0.0836	8.6141±0.5618
40	0.078	0.074	0.071	0.0743	16.4793±7.3033
80	0.064	0.069	0.060	0.0643	27.7152±2.247
100	0.058	0.052	0.049	0.053	40.4494±5.0562

Absorbance of control (A0) = 0.089

Percentage inhibition = $(A_0-A_1)/A0 \times 100$

Figure No.1: DPPH antioxidant activity of Extract



IC_{50} = 107mg/ml

Table No.2: DPPH Scavenging Assay of Standard

Concentration (Mg/Ml)	Absorbance				% Inhibition
	A1	A2	A3	AVERAGE	
10	0.099	0.096	0.097	0.09733	3.24±0.5618
20	0.082	0.093	0.090	0.088333	10.61±0.5618
40	0.079	0.094	0.091	0.088	17.47±7.3033
80	0.065	0.079	0.070	0.07133	37.7152±2.247
100	0.068	0.062	0.059	0.063	50.4494±5.0562

IC_{50} = 112 mg/ml

Hydrogen Peroxide Radical Scavenging Assay

The phenolic compounds in the *Acmella ciliata* extract likely play a role in scavenging hydrogen peroxide, which itself generates hydroxyl radicals in cells. The hydrogen peroxide scavenging activity was assessed at various concentrations: 10 mg/ml, 20 mg/ml, 40 mg/ml, 80 mg/ml, and 100 mg/ml. At 10 mg/ml: Absorbance values were 0.782, 0.795, and 0.779, with an average absorbance of 0.772 and a percentage inhibition of 3.0447 ± 0.1855. At 20 mg/ml: Absorbance values were 0.643, 0.639, and 0.635, with an average absorbance of 0.650 and a percentage inhibition of 21.1108 ± 0.4936. At 40 mg/ml: Absorbance values were 0.558, 0.571, and 0.565, with an average absorbance of 0.564 and a percentage inhibition of 30.2879 ± 0.4321. At 80 mg/ml: Absorbance values were 0.457, 0.463, and 0.462, with an average absorbance of 0.4606 and a percentage inhibition of 43.1275 ± 0.3086. At 100 mg/ml:

Absorbance values were 0.276, 0.283, and 0.269, with an average absorbance of 0.276 and a percentage inhibition of 65.7201 ± 0.4321.The control had an absorbance of 0.81, he comparative analysis between ascorbic acid and the plant extract in the hydrogen peroxide radical scavenging assay reveals significant differences in their antioxidant activities. Ascorbic Acid consistently demonstrated higher percentage inhibition across all concentrations compared to the plant extract. For instance, at 10 mg/mL, ascorbic acid showed 13.0447% inhibition, whereas the plant extract only achieved 3.0447%. At higher concentrations, this trend persisted, with ascorbic acid achieving 75.7201% inhibition at 100 mg/mL, significantly outperforming the plant extract, which reached 65.7201% at the same concentration. The IC50 value, which indicates the concentration required to achieve 50% inhibition, was lower for the plant extract (86 µg/mL) compared to ascorbic acid (104 µg/mL). This suggests that the plant extract can reach half of its maximum scavenging capacity at a lower concentration than ascorbic acid, indicating a strong potential as an antioxidant, especially at moderate concentrations. Although the plant extract demonstrates considerable antioxidant potential, particularly at concentrations around its IC50 value, ascorbic acid remains the more potent antioxidant overall. Its higher percentage inhibition across a broader range of concentrations makes it more effective in neutralizing hydrogen peroxide radicals. The plant extract could be a valuable natural alternative for antioxidant applications, especially where moderate radical scavenging is sufficient. However, in situations requiring stronger antioxidant protection, ascorbic acid would be the preferred choice due to its superior performance at higher concentrations while the plant extract shows promising antioxidant activity, especially considering its lower IC50 value, ascorbic acid outperforms the extract in terms of overall effectiveness across the tested concentrations.

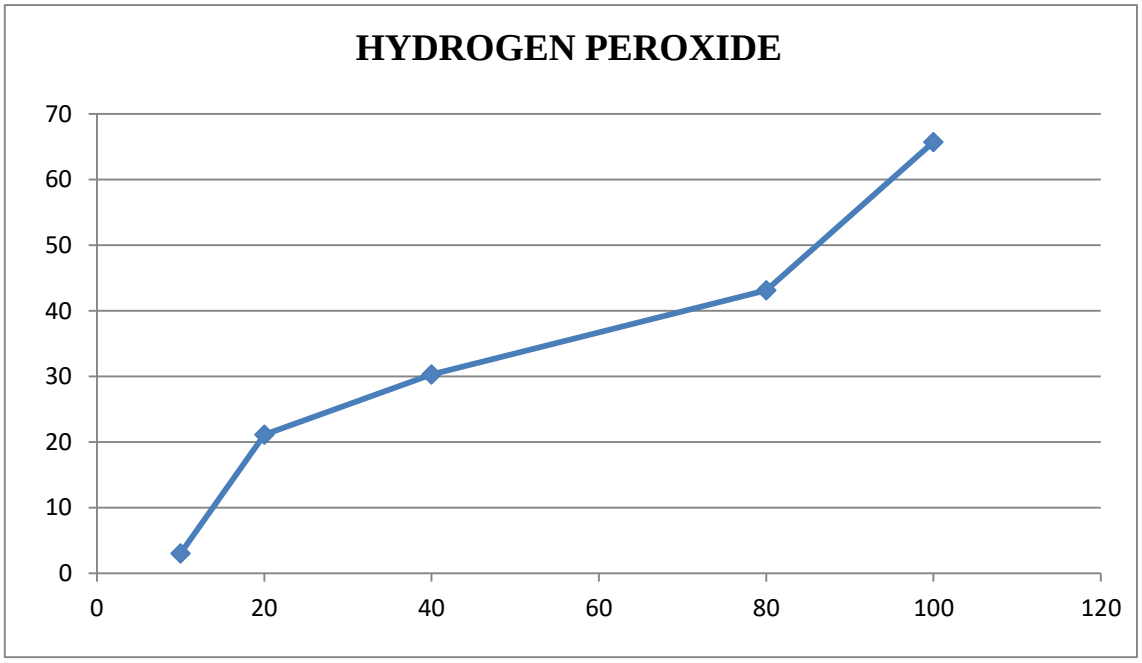
Table No.3; Hydrogen Peroxide Radical Scavenging Assay of Extract

Concentration(mg/ml)	**Absorbance**				% **Inhibition**
	A1	**A2**	**A3**	**Average**	
10	0.782	0.795	0.779	0.772	3.0447±0.1855
20	0.643	0.639	0.635	0.650	21.1108±0.4936
40	0.558	0.571	0.565	0.564	30.2879±0.4321
80	0.457	0.463	0.462	0.4606	43.1275±0.3086
100	0.276	0.283	0.269	0.276	65.7201±0.4321

Absorbance of control (A0) = 0.810

Percentage inhibition = $(A_0-A_1)/A0$ x100

Figure No.2 Hydrogen Peroxide antioxidant activity of Extract



IC_{50} = 86µg/ml

Table No.3; Hydrogen Peroxide Radical Scavenging Assay of standard

Concentration(mg/ml)	Absorbance				% Inhibition
	A1	A2	A3	Average	
10	0.882	0.895	0.879	0.872	13.0447±0.2
20	0.743	0.739	0.735	0.750	31.1108±0.5
40	0.658	0.671	0.665	0.664	41.2879±0.5
80	0.557	0.563	0.562	0.5606	53.1275±0.4
100	0.376	0.383	0.369	0.376	75.7201±0.5

IC_{50} = 104µg/ml

Ferric Ion Reducing Antioxidant Power (FRAP) Assay

The Ferric Ion Reducing Antioxidant Power (FRAP) test measures the ability of plant extracts to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This reduction process is highly reactive, particularly with oxygen and superoxide radicals. Plant secondary metabolites may possess properties that counteract the effects of nitric oxide, which can be harmful when in excess. By scavenging nitric oxide, these metabolites could help prevent harmful chain reactions initiated by excessive nitric oxide production.

The FRAP activity of the plant extract was evaluated at various concentrations: 10 mg/ml, 20 mg/ml, 40 mg/ml, 80 mg/ml, and 100 mg/ml. The absorbance values and percentage inhibition for each concentration are as follows: At 10 mg/ml, the absorbance values were 1.572, 1.549, and 1.509, with an average absorbance of 1.543 and a percentage inhibition of 30.2373 ± 4.639.

At 20 mg/ml, the absorbance values were 1.412, 1.443, and 1.397, with an average absorbance of 1.417 and a percentage inhibition of 37.9929 ± 0.9641. At 40 mg/ml, the absorbance values were 1.264, 1.259, and 1.237, with an average absorbance of 1.253 and a percentage inhibition of 45.0904 ± 0.5916. At 80 mg/ml, the absorbance values were 1.123, 1.102, and 1.129, with an average absorbance of 1.118 and a percentage inhibition of 51.0065 ± 0.1318. At 100 mg/ml, the absorbance values were 0.708, 0.866, and 0.954, with an average absorbance of 0.843 and a percentage inhibition of 65.1350 ± 5.2973. The absorbance of the control (A0) was 2.282. In summary, the comparative analysis of the ferric ion reducing antioxidant power between ascorbic acid and the plant extract highlights their distinct advantages and potential applications based on their antioxidant efficacy. The plant extract exhibits a lower IC50 value of 32 mg/mL, indicating that it achieves 50% inhibition at a lower concentration compared to ascorbic acid. This lower IC50 suggests that the plant extract is relatively more efficient at scavenging free radicals and reducing ferric ions even at modest concentrations.: Due to its lower IC50, the plant extract is potentially more effective in formulations where lower concentrations are preferred. This can be advantageous in applications where a cost-effective or less concentrated antioxidant is desired, without compromising efficacy Ascorbic acid, with an IC50 value of 105 mg/mL, demonstrates strong antioxidant activity, particularly at higher concentrations. It effectively reduces ferric ions and exhibits significant inhibition percentages at elevated doses.Ascorbic acid's higher IC50 indicates that it is suitable for applications requiring potent antioxidant effects, especially when higher concentrations are feasible or necessary. Its robust performance at higher concentrations makes it ideal for scenarios demanding maximum antioxidant activity.Both the plant extract and ascorbic acid offer valuable antioxidant properties, but their applications differ based on concentration and potency:Its lower IC50 value makes it a preferable choice for formulations needing effective antioxidant protection at lower concentrations. This can be particularly useful in products where a lower concentration of antioxidant is desired, either for cost reasons or to achieve a specific formulation balance.Despite requiring higher concentrations to achieve its antioxidant effects, it remains a potent and reliable antioxidant. It is particularly well-suited for applications where high levels of antioxidant activity are required, such as in high-potency supplements or formulations designed for intensive antioxidant protection. In essence, the choice between the plant extract and ascorbic acid will depend on the specific requirements of the application, including the desired concentration of antioxidant and the level of potency needed. Both substances possess significant antioxidant potential, but their optimal use will align with their respective strengths in reducing ferric ions and neutralizing free radicals.

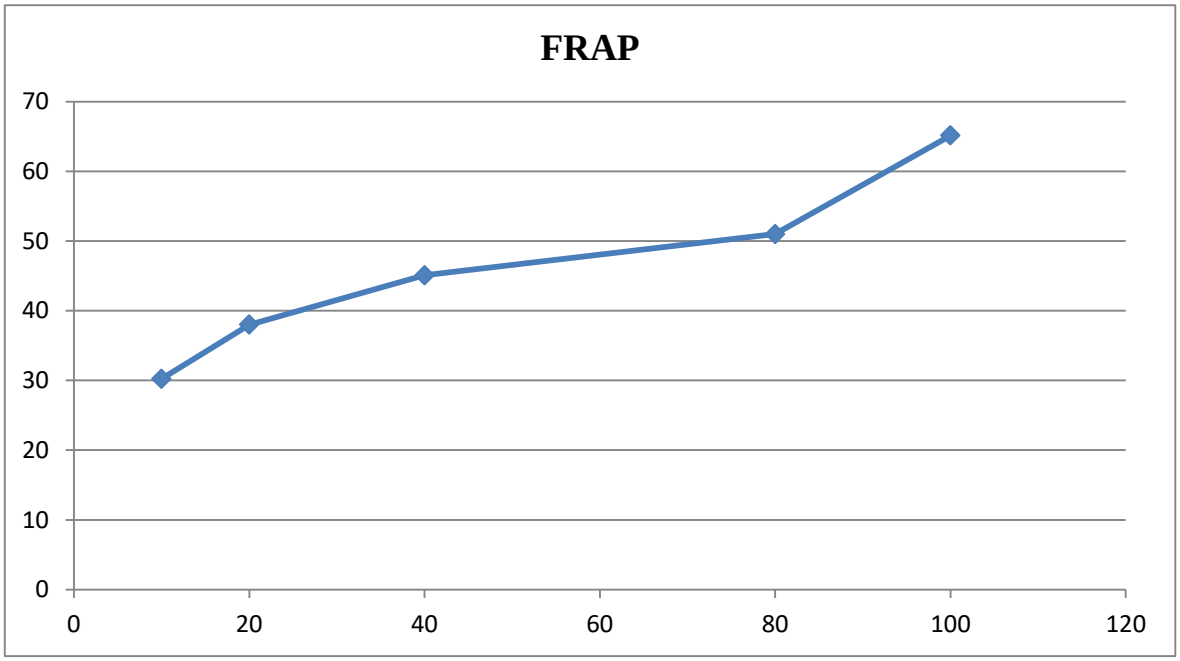
Table No.4 Ferric Ion Reducing Antioxidant Power

Concentration(mg/ml)	Absorbance				% Inhibition
	A1	A2	A3	Average	
10	1.572	1.549	1.509	1.543333	30.2373±4.639
20	1.412	1.443	1.397	1.417333	37.9929±0.9641
40	1.264	1.259	1.237	1.253333	45.0904±0.5916
80	1.123	1.102	1.129	1.118	51.0065±0.1318
100	0.708	0.866	0.954	0.842667	65.1350±5.2973

Absorbance of control (A0) = 2.282

Percentage inhibition = $(A_0-A_1)/A0 \times 100$

Figure No.3 Ferric Ion Reducing Antioxidant Power



IC_{50} = 32mg/ml

Table No.4 Ferric Ion Reducing Antioxidant Power of standard

Concentration(mg/ml)	Absorbance				% Inhibition
	A1	A2	A3	Average	
10	0.78	0.79	0.7	08	4.04±0.185
20	0.643	0.63	0.6	0.6	22.11±0.493
40	0.558	0.57	0.56	0.56	30.28±0.432
80	0.457	0.46	0.46	0.46	42.12±0.308
100	0.276	0.28	0.26	0.27	66.72±0.432

IC_{50} = 107mg/ml

MTT Assay

The MTT assay was performed to evaluate the anticancer activity of the plant extract on a specified cell line. The assay measures cell viability based on the reduction of MTT, a yellow tetrazole, to purple formazan by mitochondrial enzymes in viable cells. The optical density (OD) values at 540 nm were recorded for various concentrations of the plant extract, and the percentage viability was calculated. The decrease in cell viability suggests that the plant extract possesses anticancer properties, as it can inhibit the growth of cancer cells in a dose-dependent manner. The significant reduction in cell viability at higher concentrations (50 and 100 µg/ml) highlights the potential of the plant extract as a source of anticancer agents. Further studies, such as apoptosis assays, cell cycle analysis, and molecular mechanism studies, are needed to elucidate the exact pathways through which the plant extract exerts its anticancer effects. Additionally, in vivo studies and clinical trials would be necessary to confirm the efficacy and safety of the plant extract in treating cancer. The MTT assay results demonstrate that the plant extract exhibits a dose-dependent reduction in cancer cell viability. At the highest concentration (100 µg/ml), the cell viability decreased to approximately 80.49%, suggesting a significant cytotoxic effect. Even at lower concentrations (6.25 and 12.5 µg/ml), the plant extract showed a reduction in cell viability compared to the control, indicating potential anticancer properties. he results suggest that the plant extract contains bioactive compounds capable of inhibiting cancer cell growth. The reduction in viability at higher concentrations highlights the potency Demonstrates a gradual decrease in cell viability as the concentration increases, with a percentage viability ranging from 96.25% at 6.25 µg/mL to 80.49% at 100 µg/mL. This indicates a dose-dependent reduction in cell viability, reflecting the plant extract's potential anticancer activity.Cyclophosphamide (Standard) Also shows a reduction in cell viability with increasing concentration, ranging from 97.25% viability at 6.25 µg/mL to 81.49% at 100 µg/mL. Cyclophosphamide's performance is comparable to that of the plant extract, demonstrating its effectiveness as an anticancer agent. At lower concentrations (6.25 and 12.5 µg/mL), the plant extract shows slightly lower efficacy in reducing cell viability compared to cyclophosphamide. The percentage viability at these concentrations is higher compared to the standard, suggesting that the extract may be less potent at lower doses Shows slightly better effectiveness at lower concentrations, with a more pronounced reduction in cell viability compared to the plant extract. This suggests that cyclophosphamide is more potent at lower doses. Both the plant extract and cyclophosphamide exhibit similar trends in reducing cell viability at higher concentrations (50 and 100 µg/mL). The percentage viability is comparable, indicating that the plant extract and cyclophosphamide have similar potency at higher doses. Although less effective than cyclophosphamide at lower concentrations; it shows comparable anticancer activity at higher concentrations. This suggests that while the plant extract may require higher doses to achieve similar effects as cyclophosphamide, it still holds significant potential as an anticancer agent. Consistently demonstrates strong anticancer activity across all concentrations, particularly at lower doses. It serves as a potent standard, indicating its well-established efficacy in cancer treatment. Both the plant extract and cyclophosphamide exhibit anticancer activity, with cyclophosphamide showing slightly better performance, especially at lower concentrations.

The plant extract, while showing similar trends in reducing cell viability at higher concentrations, may require higher doses to match the efficacy of cyclophosphamide. These results highlight the potential of the plant extract as an effective anticancer agent, with further investigation needed to optimize its dosage and efficacy.

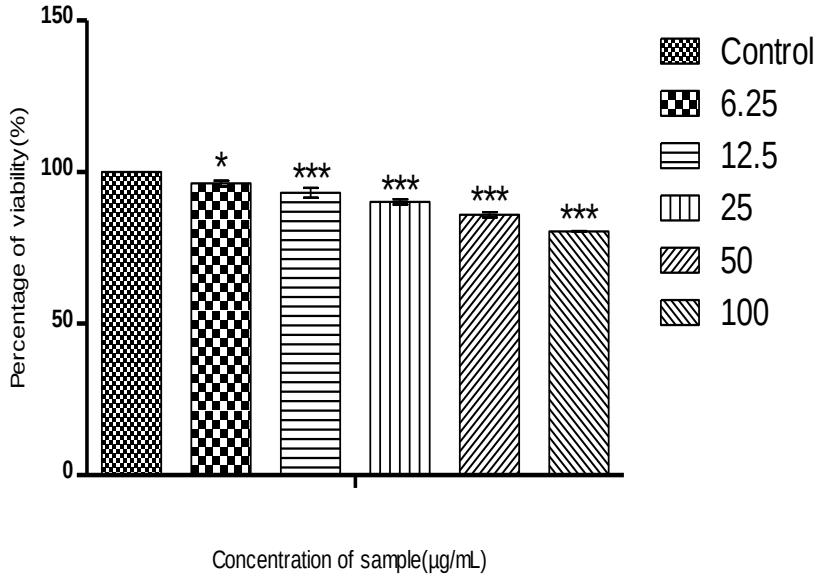
Table No.6: MTT Assay OD Value Involvement

Sample Concentration (µg/ml)	**OD I**	**OD II**	**OD III**	**Average Absorbance @ 540nm**	**Percentage Viability**
Control	0.6836	0.6984	0.7019	0.6946	100.00
6.25	0.67	0.6629	0.6728	0.6686	96.25
12.5	0.6596	0.6412	0.6409	0.6472	93.18
25	0.6226	0.6352	0.6215	0.6264	90.19
50	0.5959	0.6025	0.5915	0.5966	85.90
100	0.5509	0.5615	0.5648	0.5591	80.49
Standard					
6.25	0.57	0.5629	0.5728	0.5686	97.25
12.5	0.5596	0.5412	0.5409	0.5472	94.18
25	0.5226	0.5352	0.5215	0.5264	91.19
50	0.4959	0.5025	0.4915	0.5966	86.90
100	0.4509	0.4615	0.4648	0.5591	81.49

Table No.7: MTT Assay Statistics

Cell line -									
Sample									
	OD1	**OD2**	**OD3**	**Percentage viability 1**	**Percentage viability 2**	**Percentage viability 3**	**Average**	**Stdev**	**Std error**
Control	0.6836	0.6984	0.7019	100	100	100	100	0	0
6.25	0.67	0.6629	0.6728	98.0105	94.917	95.8541	96.2605	1.58633	0.91587
12.5	0.6596	0.6412	0.6409	96.4892	91.8099	91.3093	93.2028	2.85709	1.64954
25	0.6226	0.6352	0.6215	91.0767	90.9507	88.5454	90.1909	1.42648	0.82358
50	0.5959	0.6025	0.5915	87.1709	86.2686	84.2713	85.9036	1.48386	0.85671
100	0.5509	0.5615	0.5648	80.5881	80.3981	80.4673	80.4845	0.09616	0.05552
Standard									
6.25	0.57	0.5629	0.5728	97.25	97.25	96.9	97.25	1.6	0.9
12.5	0.5596	0.5412	0.5409	94.17	94.18	92.3	94.18	2.9	1.7
25	0.5226	0.5352	0.5215	91.18	91.19	89.6	91.19	1.5	0.9
50	0.4959	0.5025	0.4915	86.9	86.90	85.3	86.90	1.5	0.9
100	0.4509	0.4615	0.4648	81.5	81.49	81.5	81.49	0.6	0.1

Figure No.5: MTT Assay



SUMMARY AND CONCLUSION

The whole plant of *Acmella ciliata* were collected, dried, extracted and subjected to prescribed in-vitro antioxidant studies. In conclusion, the results obtained in the present study indicated that the hydroethanolic whole plant extract of *Acmella ciliate* have appreciable antioxidant capacity. Hence, this can be used as natural sources of antioxidants as they could have great importance as therapeutic agents in slowing or preventing the progress of aging and oxidative stress related degenerative diseases and cancer. Further studies that are aimed at isolating and characterizing the pure phytoactive principles for enhancement of anti oxidant property are recommended. These findings provide a strong basis for further investigation into the specific compounds responsible for the anticancer activity and their mechanisms of action.To fully establish the therapeutic potential of *Acmella ciliata*, additional studies are necessary. These should include: Apoptosis assays to determine if the extract induces programmed cell death. Cell cycle analysis to understand the impact of the extract on cell division. Molecular studies to elucidate the pathways involved in the extract's anticancer effects.In vivo studies and clinical trials to evaluate the efficacy and safety of the extract in living organisms.The hydroalcoholic extract of *Acmella ciliata* exhibits significant antioxidant and anticancer activities in vitro. These findings provide a strong basis for further investigation into the plant's therapeutic potential. The results suggest that *Acmella ciliata* could serve as a natural source of antioxidants, potentially useful in slowing down aging and preventing oxidative stress-related diseases, including cancer. However, to fully establish its therapeutic potential, more extensive studies, including apoptosis assays, cell cycle analysis, molecular studies, in vivo experiments, and clinical trials, are necessary. Overall, *Acmella ciliata* holds promise as a natural therapeutic agent, and continued research could lead to its development as an effective treatment for cancer and other diseases linked to oxidative stress.

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