**Anti-Cancer Potential of Blueberry Extract against Colon Cancer Cell Lines**

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**ABSTRACT:**

Colon cancer remains one of the most prevalent malignancies worldwide, with rising incidences in both developed and developing nations. Conventional treatments for colon cancer, such as chemotherapy and radiotherapy, often produce adverse side effects, necessitating the exploration of natural alternatives. This study investigates the anti-cancer activity of acetone fruit extract of Vaccinium pallidum (blueberry) against colon cancer cell lines (HCT116, HT29, and HT115) using the MTT assay. The extract was prepared and screened for phytochemicals, revealing the presence of alkaloids, flavonoids, tannins, saponins, phenolics, carbohydrates, phytosterols, and triterpenoids, which are known for their antioxidant and anti-cancer properties.

Cytotoxicity studies demonstrated dose-dependent activity, with CTC50 values of 230 ± 16.32 µg/ml, 140 ± 23.0 µg/ml, and 290 ± 26 µg/ml for HCT116, HT29, and HT115 cell lines, respectively. Higher cytotoxicity was observed in HT29 cells, indicating stronger inhibitory potential. The results suggest that the extract may induce apoptosis and inhibit proliferation through mitochondrial activity disruption.

This study highlights the therapeutic promise of Vaccinium pallidum in combating colon cancer, underscoring the importance of dietary phytochemicals as safe, effective alternatives. Future research should focus on elucidating the precise molecular pathways involved and evaluating in vivo efficacy to support clinical applications.

**KEYWORDS:** Colon Cancer, Blueberry Extract, Vaccinium pallidum, Cytotoxicity, Phytochemicals, MTT Assay

**INTRODUCTION:**

Colon cancer is one of the most prevalent forms of cancer in both men and women and is considered the second leading cause of cancer-related deaths globally, particularly in developed nations such as the United States. The risk of developing colon cancer increases significantly with age, making it more common in older populations. While colon cancer is the most frequent malignancy affecting the colon, other rare forms of cancer, such as lymphoma, carcinoid tumors, melanoma, and sarcomas, can also occur in this region. Typically, colon cancer originates in the lining of the colon, often beginning as benign polyps that gradually develop into malignant tumors if left untreated. The exact cause of colon cancer remains unclear, though lifestyle factors, genetic predispositions, and aging are major contributors to its development [1-2].

Restricted colon cancer, where the disease is confined to the colon wall, can often be effectively treated with surgery. However, advanced stages that spread beyond the colon are generally incurable, with treatment options like chemotherapy extending survival and improving the quality of life. Early detection methods, such as colonoscopy, play a critical role in managing the disease. According to the World Health Organization, colorectal cancer (including colon cancer) remains the third most common cancer worldwide, with over 1.93 million new cases and 935,000 deaths reported in 2020. Colon cancer is notably more prevalent in developed countries due to factors such as dietary habits, sedentary lifestyles, and aging populations [3-4].

The molecular basis of colon cancer highlights the role of genetic mutations. A mutation in the *APC* gene, a tumor suppressor, is frequently observed in colon cancer. The *APC* protein regulates the levels of β-catenin, a protein involved in cell signaling and gene transcription. When the *APC* gene is mutated, β-catenin accumulates excessively and translocates to the nucleus, where it activates genes that drive cancer development. Additionally, mutations in oncogenes such as *KRAS*, *RAF*, and *PI3K* contribute to unchecked cell proliferation by overactivating growth factor signaling pathways. Mutations in tumor suppressor genes like *TGF-β* and *DCC* are also commonly associated with colon cancer [5]. For instance, *TGF-β* signaling is disrupted in nearly half of colon cancer cases, and deletions in the *DCC* gene often occur, further promoting tumor progression [6].

Blueberries, known for their potent antioxidant properties, have been identified as a potential dietary intervention in cancer prevention. Their primary function lies in protecting cells from damage caused by reactive oxygen species (ROS), commonly referred to as free radicals. These unstable molecules play a significant role in the onset and progression of chronic diseases, including cancer and cardiovascular diseases. Blueberries exhibit one of the highest antioxidant activities among fruits and vegetables, making them a valuable dietary source for combating oxidative stress. By neutralizing free radicals, antioxidants from blueberries may reduce the risk of cancer and other oxidative stress-related conditions. The inclusion of antioxidant-rich foods like blueberries in the diet has the potential to complement therapeutic strategies and promote overall health [7-10].

**MATERIALS AND METHODS:**

**MATERIALS:**

The following materials were used in the study: 3-(4,5-dimethylthiazol-2-yl)-5-diphenyltetrazolium bromide (MTT), fetal bovine serum (FBS), phosphate-buffered saline (PBS), Dulbecco’s Modified Eagle’s Medium (DMEM), and trypsin, all procured from Sigma Aldrich, St. Louis, USA. Ethylenediaminetetraacetic acid (EDTA), glucose, and antibiotics were sourced from HiMedia Laboratories, Mumbai, India. Additionally, dimethyl sulfoxide (DMSO) and propanol were obtained from E. Merck, Mumbai, India.

**METHODS:**

**Extraction of plant material:**

The powdered plant material (100 g) was extracted using 70% acetone in deionized water. The mixture was processed in a stainless steel laboratory blender with cooling overnight. The chilled extract was filtered through five layers of cheesecloth. The filtrate was then centrifuged for 10 minutes, and the supernatant was transferred to a Pyrex baking dish. The dish was placed under a fume hood to allow the acetone to evaporate [11].

**Phytochemical Analysis of Acetone Extract from Blueberry (Vaccinium myrtillus):**

The acetone extract derived from blueberry (Vaccinium myrtillus) was subjected to qualitative phytochemical screening to identify the presence of various bioactive compounds. The procedures used for the detection of alkaloids, carbohydrates, flavonoids, fixed oils, phytosterols, glycosides, proteins, tannins, triterpenoids, and amino acids are described below [12].

**i. Alkaloid Detection Tests:**

1. **Mayer’s Test:** A small portion of the extract was mixed with dilute hydrochloric acid and filtered. To 1 ml of the filtrate, a few drops of Mayer’s reagent (potassium mercuric iodide) were added. The formation of a cream-colored precipitate indicated the presence of alkaloids.
2. **Dragendorff’s Test:** The filtrate was treated with Dragendorff’s reagent (potassium bismuth iodide). The appearance of an orange-red precipitate suggested the presence of alkaloids.
3. **Wagner’s Test:** A few drops of Wagner’s reagent (iodine in potassium iodide) were added to the filtrate. The formation of a brown precipitate confirmed the presence of alkaloids.
4. **Hager’s Test:** Hager’s reagent (saturated picric acid solution) was added to the filtrate. The development of an orange-yellow precipitate indicated alkaloid presence [13].

**ii. Carbohydrate Detection Tests:**

1. **Molisch’s Test:** A few drops of Molisch’s reagent (α-naphthol) were added to the extract solution. Concentrated sulfuric acid was slowly added along the side of the test tube. The appearance of a purple ring at the interface suggested carbohydrates.
2. **Barfoed’s Test:** The extract solution was treated with Barfoed’s reagent (copper acetate solution) and heated in a water bath. A red precipitate indicated the presence of monosaccharides.
3. **Benedict’s Test:** Benedict’s reagent (copper sulfate, sodium carbonate, and sodium citrate) was mixed with the extract and heated. A reddish-brown precipitate confirmed the presence of reducing sugars.
4. **Fehling’s Test:** The extract solution was combined with equal volumes of Fehling’s solution A (copper sulfate) and Fehling’s solution B (potassium tartrate and sodium hydroxide) and heated. The formation of a brick-red precipitate indicated reducing sugars [14].

**iii. Flavonoid Detection Tests:**

1. **Lead Acetate Test:** The extract was treated with lead acetate solution. A yellow precipitate signified the presence of flavonoids.
2. **Sodium Hydroxide Test:** The extract was treated with increasing amounts of sodium hydroxide. A yellow coloration that disappeared upon the addition of acid confirmed the presence of flavonoids [15].

**iv. Fixed Oil Detection Tests:**

1. **Spot Test:** A small quantity of the extract was pressed between two filter papers. The appearance of an oily stain indicated the presence of fixed oils.
2. **Saponification Test:** The extract was mixed with alcoholic potassium hydroxide and phenolphthalein, and then heated in a water bath. Soap formation confirmed the presence of fixed oils [16].

**v. Phytosterol Detection Tests:**

1. **Salkowski Test:** The extract was dissolved in chloroform, and concentrated sulfuric acid was added. A reddish-brown coloration in the lower layer indicated phytosterols.
2. **Liebermann-Burchard Test:** The extract was mixed with acetic anhydride, and sulfuric acid was added dropwise along the side of the test tube. A brown ring at the junction and a green upper layer indicated the presence of Phytosterols [17].

**vi. Glycoside Detection Tests:**

1. **Legal’s Test:** The extract was hydrolyzed with hydrochloric acid, followed by the addition of pyridine and sodium nitroprusside. Sodium hydroxide was added to alkalize the solution. The development of a pink to yellow color indicated glycosides.
2. **Borntrager’s Test:** The hydrolyzed extract was treated with chloroform. The chloroform layer was separated and treated with dilute ammonia. The appearance of a pink color indicated glycosides [18].

**vii. Protein Detection Tests:**

1. **Millon’s Test:** The extract solution was treated with Millon’s reagent and heated. A red coloration indicated the presence of proteins.
2. **Biuret Test:** The extract was treated with sodium hydroxide and dilute copper sulfate. The appearance of a violet or purple color indicated proteins [19].

**viii. Tannin Detection Tests:**

1. **Ferric Chloride Test:** The extract was treated with 5% ferric chloride solution. A blue-black coloration confirmed the presence of tannins.
2. **Lead Acetate Test:** The extract was treated with lead acetate solution. A white precipitate suggested tannins.
3. **Bromine Water Test:** Bromine water was added to the extract. Discoloration indicated the presence of tannins [20].

**ix. Triterpenoid Detection Tests:**

1. **Salkowski Test:** Concentrated sulfuric acid was added to the extract dissolved in chloroform. A golden yellow layer indicated the presence of triterpenoids.
2. **Liebermann-Burchard Test:** Acetic anhydride was added to the extract solution followed by concentrated sulfuric acid. A red color in the lower layer confirmed the presence of Triterpenoids [21].

**x. Amino Acid Detection Tests:**

1. **Ninhydrin Test:** The extract solution was treated with ninhydrin reagent. The formation of a blue color indicated the presence of amino acids [22].

**Evaluation of anti-cancer activity:**

**Cell Lines and Culture Medium:**

Human colorectal cancer cell lines (HCT116, HT115, and HT-29) were sourced from the National Centre for Cell Sciences (NCCS), Pune, India. These cells were maintained in Dulbecco’s Modified Eagle Medium (DMEM) enriched with 10% inactivated fetal bovine serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml), and amphotericin B (5 µg/ml). The cells were incubated in a humidified environment with 5% CO₂ at 37°C until confluence was achieved. For subculturing, cells were detached using TPVG solution (containing 0.2% trypsin, 0.02% EDTA, and 0.05% glucose in phosphate-buffered saline). Cells were propagated in 25 cm² culture flasks, while experimental procedures were conducted using 96-well microplates [23].

**Preparation of Test Solutions:**

For cytotoxicity evaluation, test compounds were accurately weighed, dissolved in dimethyl sulfoxide (DMSO), and diluted with DMEM containing 2% inactivated FBS to prepare a 1 mg/ml stock solution. The solution was sterilized through filtration. Serial dilutions (two-fold) were performed to obtain various concentrations required for cytotoxicity assays [24].

**MTT Assay for Cell Viability:**

The MTT assay evaluates cell viability based on mitochondrial activity. Viable cells reduce MTT, a yellow tetrazolium dye, into formazan, a blue crystalline product, through the action of mitochondrial succinate dehydrogenase. The intensity of the resulting color is proportional to the number of living cells and their metabolic activity [25].

**Experimental Procedure:**

Monolayer cultures were trypsinized, and cell density was adjusted to 1 × 10⁵ cells/ml in DMEM supplemented with 10% FBS. Approximately 10,000 cells (0.1 ml) were seeded into each well of a 96-well microplate. After 24 hours, the supernatant was removed, and the wells were washed with fresh medium. Test compounds at varying concentrations (100 µl) were added to the wells containing the partial monolayer. The plates were incubated at 37°C in a 5% CO₂ atmosphere for 72 hours, with observations recorded every 24 hours.

Following the incubation period, the test solutions were aspirated, and 50 µl of MTT solution (prepared in PBS) was introduced into each well. The plates were incubated for an additional 3 hours. After this, the supernatant was discarded, and 100 µl of propanol was added to dissolve the formazan crystals. Absorbance was measured at 540 nm using a microplate reader. Cell growth inhibition was calculated, and the concentration required to achieve 50% inhibition (IC₅₀) was determined from dose-response curves [26-30].

Mean OD test group

X 100

Mean OD of control group

% Growth Inhibition = 100 –

**RESULTS:**

**Percentage yield of fruit extract:**

The yield obtained from the Acetone fruit extract of vacciniumpallidum (F) Blueberry. Was about 162 grams (13.5 %) from 1.2 kg leaves.

**Identification of phytochemical analysis:**

The phytochemical analysis of the acetone extract of Vaccinium pallidum (blueberry) revealed the presence of various bioactive compounds essential for therapeutic applications. Qualitative tests confirmed the presence of carbohydrates, saponins, alkaloids, flavonoids, phenolics, tannins, phytosterols, and triterpenoids, while cardiac glycosides and fixed oils were absent. Carbohydrates were identified through Molisch’s, Barfoed’s, Benedict’s, and Fehling’s tests, indicating the presence of reducing sugars, which may contribute to the metabolic activity of cells. Alkaloids were detected by Mayer’s, Dragendorff’s, Wagner’s, and Hager’s tests, suggesting anti-cancer and anti-inflammatory properties. Flavonoids, confirmed using lead acetate and sodium hydroxide tests, exhibit antioxidant activity, free radical scavenging, and enzyme inhibition. Phenolics and tannins, detected via ferric chloride, lead acetate, and bromine water tests, are known for their antioxidant and DNA-protective properties. Phytosterols and triterpenoids, confirmed by Salkowski and Liebermann-Burchard tests, provide anti-inflammatory and membrane-stabilizing activities. Saponins, identified through froth formation, contribute to membrane permeability and apoptosis (Table 1).

**Table 1: Phytochemical analysis Acetone fruit extract of vacciniumpallidum (F) Blue berry**

|  |  |  |  |
| --- | --- | --- | --- |
| **Phytochemical Test** | **Test Method** | **Result** | **Interpretation** |
| **Test for Carbohydrates** | Molisch’s Test | **+** | Indicates the presence of carbohydrates, confirmed by a purple or reddish ring. |
| **Test for Glycosides** | Modified Borntrager’s Test, Keller-Killiani Test | **-** | Indicates the absence of glycosides in the sample. |
| **Test for Saponins** | Foam Test | **+** | Persistent froth formation confirms the presence of saponins. |
| **Test for Alkaloids** | Mayer’s Test, Dragendorff’s Test | **+** | Formation of precipitates confirms the presence of alkaloids. |
| **Test for Flavonoids** | Alkaline Reagent Test | **+** | Yellow coloration confirms the presence of flavonoids. |
| **Test for Phenolics and Tannins** | Ferric Chloride Test, Tannin Test | **+** | Blue-black or greenish coloration indicates phenolics and tannins are present. |
| **Test for Phytosterols and Triterpenoids** | Liebermann-Burchard Test, Salkowski Test | **+** | Positive results confirm the presence of phytosterols and triterpenoids. |
| **Test for Fixed Oils and Fats** | Oily Spot Test | **-** | Absence of translucent spots confirms no fixed oils or fats are present. |

The phytochemical analysis of the acetone fruit extract of Vaccinium pallidum (blueberry) revealed several key observations. The presence of carbohydrates suggests their potential role as bioactive components contributing to energy metabolism. Saponins were detected, which are known for their ability to induce apoptosis in cancer cells and enhance membrane permeability. Alkaloids were also present, exhibiting potential anti-cancer properties by disrupting cellular division and DNA synthesis. Flavonoids and phenolics, identified in the extract, act as strong antioxidants, contributing to free radical scavenging and providing significant anti-inflammatory activities. Additionally, phytosterols and triterpenoids were found, which are known for their anti-inflammatory and anticancer properties, further enhancing the extract’s therapeutic potential. Notably, the absence of glycosides and fixed oils reduces the likelihood of adverse effects often associated with these compounds, making the extract potentially safer for therapeutic applications. This phytochemical profile highlights the extract's promising pharmacological potential.

**In Vitro Anti-cancer activity:**

**MTT Assay:**

The MTT assay evaluated the cytotoxic potential of the acetone extract against three colon cancer cell lines—HCT116, HT29, and HT115. The results showed dose-dependent effects, with CTC50 values calculated for each cell line. For HCT116, cytotoxicity ranged from 20.11% at 31.2 µg/ml to 69.49% at 500 µg/ml, with a CTC50 value of 230 ± 16.32 µg/ml. HT29 cells exhibited higher sensitivity, with cytotoxicity ranging from 22.36% at 31.2 µg/ml to 86.50% at 500 µg/ml, and a CTC50 value of 140 ± 23.0 µg/ml. HT115 cells showed relatively lower sensitivity, with cytotoxicity ranging from 6.11% at 31.2 µg/ml to 67.44% at 500 µg/ml, and a CTC50 value of 290 ± 26 µg/ml.

**Cytotoxic Properties of the Test Drug Against HCT116 Cell Lines:**

The cytotoxic potential of the acetone fruit extract of *Vaccinium pallidum* (F) blueberry was evaluated using the MTT assay on HCT116 cell lines. The half-maximal cytotoxic concentration (CTC50) values are presented in Table 2.

**Table 2: CTC 50 value of Acetone fruit extract of vacciniumpallidum (F) Blue berry**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr. No** | **Name of cell line** | **Test concentration** | **% of cytotoxicity** | **CTC 50****( µg/ml)** |
| 1 | HCT116 | 500 | 69.49±1.30 | 230±16.32\* |
| 2 | 250 | 52.21± 0.23 \* |
| 3 | 125 | 41.81 ±1.64 |
| 4 | 62.5 | 31.91 ±14.73 \* |
| 5 | 31.2 | 20.11± 4.21\* |

**Acetone fruit extract of Vacciniumpallidum (F) Blue berry:**

**Cytotoxic assay on HCT116 cell line:**

The results of Invitrocytotoxic concentration of Acetone fruit extract of vacciniumpallidum (F) Blue berry in MTT Assay method on HCT 116 cell lines. CTC 50 values are given Figure 1.

**Concentration in µg/ml**

0

CONTROL

31.2

61.5

125

250

500

10

0

20.11

20

31.91

30

41.81

40

52.21

60

50

69.49

80

70

**HCT 116**

**Figure 1: Graphical representation of cytotoxicity of Acetone fruit extract of vacciniumpallidum (F) Blue berry with CTC 50**

**Cytotoxic properties of test drug against HT-29 cell lines:**

The result of In vitro cytotoxic concentration of Acetone fruit extract of vacciniumpallidum (F) Blue berry in MTT Assay method on HT29 cell lines. CTC 50 values are given in Table 3.

**Table 3: CTC 50 value of Acetone fruit extract of vacciniumpallidum (F) Blue berry**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr. No** | **Name of cell line** | **Test concentration** | **%of cytotoxicity** | **CTC 50****( µg/ml)** |
| 1 | HT- 29 | 500 | 86.50±0.86 | 140.33±23.0\*\* |
| 2 | 250 | 75.94±0.41\* |
| 3 | 125 | 48.01±8.52 |
| 4 | 62.5 | 37.45±9.87\* |
| 5 | 31.2 | 22.36±4.54 |

**Acetone fruit extract of Vacciniumpallidum (F) Blue berry:**

**Cytotoxic assay on HT-29:**

The result of In vitro cytotoxic concentration of Acetone fruit extract of vacciniumpallidum (F) Blue berry in MTT Assay method on HT29 cell lines as shown in Figure 2.

**HT29**

100

90

80

70

60

50

40

30

20

10

0

86.5

75.94

48.01

37.45

22.36

500 250 125 61.5 31.2

0

CONTROL

**Concentration in µg/ml**

**Figure 2: Graphical representation of cytotoxicity of Acetone fruit extract of vacciniumpallidum (F) Blue berry with CTC 50**

**Cytotoxic properties of test drug against HT115 cell lines:**

The results of In vitro cytotoxic concentration of Acetone fruit extract of vacciniumpallidum (F) Blue berry in MTT Assay method on HT115 cell lines as shown in Table 4.

**Table 4: CTC 50 value of Acetone fruit extract of Vacciniumpallidum (F) Blue berry**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr. No.** | **Name of cell line** | **Test concentration** | **% of****cytotoxicity** | **CTC 50****( µg/ml)** |
| 1 | HT115 | 500 | 67.44 ±0.66 | 290 ±26\* |
| 2 | 250 | 33.32 ±2.83\* |
| 3 | 125 | 21.38 ±3.88 |
| 4 | 62.5 | 8.35 ±5.22\* |
| 5 | 31.2 | 6.11 ±7.07 |

**Acetone fruit extract of Vacciniumpallidum (F) Blue berry:**

**Cytotoxic assay on HT115 Cell line:**

The result of Invitrocytotoxic concentration of Acetone fruit extract of vacciniumpallidum (F) Blue berry in MTT Assay method on HT115 cell lines as shown in Figure 3.

**HT115**

80

70

60

50

40

30

20

10

0

500

250

125

61.5

31.2

0

CONTROL

**Concentration in µg/ml**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |  |  |  |  |
|  | 67.44 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  | 33.32 |  |  |  |  |  |  |  |  |
|  |  |  |  |  | 21.38 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 8.35 |  | 6.11 |  |  |

**Figure 3: Graphical representation of cytotoxicity of Acetone fruit extract of vacciniumpallidum (F) Blue berry with CTC 50**

**DISCUSSION:**

The phytochemical profile indicates the presence of compounds with potential anti-cancer activities. Flavonoids and phenolics are reported to neutralize reactive oxygen species, reducing oxidative damage and triggering apoptosis in cancer cells. Tannins and saponins enhance membrane permeability, leading to cell lysis, while alkaloids disrupt DNA synthesis and mitotic activity. These bioactive compounds may act synergistically to inhibit cell proliferation and induce cell death.

The MTT assay results highlight dose-dependent cytotoxicity, suggesting mitochondrial dysfunction induced by the extract. The reduction of MTT to formazan requires mitochondrial enzyme succinate dehydrogenase, indicating cellular metabolism disruption. For HCT116 cells, moderate sensitivity may be attributed to mutations in the APC gene and the Wnt/β-catenin pathway, which regulate cell growth and differentiation. The extract potentially modulates these pathways, suppressing proliferation. HT29 cells demonstrated high sensitivity, possibly due to defects in DNA repair genes and overexpression of KRAS and PI3K pathways, which are effectively targeted by the extract. HT115 cells exhibited lower sensitivity, suggesting limited receptor binding or metabolic activity, necessitating higher concentrations for efficacy.

Flavonoids and phenolics in the extract act as potent antioxidants, scavenging free radicals and reducing oxidative stress. This mechanism is critical in preventing mutations and promoting apoptosis. Tannins further contribute by interacting with DNA, inhibiting transcriptional activity, and disrupting cellular integrity. Compared to conventional chemotherapeutics, the blueberry extract offers a natural alternative with reduced side effects. Its selective cytotoxicity against cancer cells supports its use as an adjunct therapy. However, further studies are needed to establish its efficacy in vivo and determine optimal dosages.

This study suggests that dietary antioxidants like blueberries could play a significant role in cancer prevention and treatment. Investigating molecular pathways, such as apoptosis, autophagy, and angiogenesis, can help elucidate the mechanisms underlying cytotoxicity. Additionally, combining the extract with existing drugs may enhance therapeutic outcomes.

While the in vitro results are promising, translating these findings to clinical applications requires detailed pharmacokinetic and pharmacodynamic studies. Challenges include bioavailability, stability, and formulation optimization for targeted delivery. This study demonstrates the anti-cancer potential of Vaccinium pallidum extract, particularly against HT29 cells, through phytochemical and cytotoxic analyses. The results validate its role as a natural source of antioxidants and anti-cancer agents. Future studies should focus on mechanistic insights, in vivo validations, and clinical trials to establish it as a viable treatment option.

**CONCLUSION:**

This study demonstrates the significant anti-cancer potential of *Vaccinium pallidum* (blueberry) extract against colon cancer cell lines (HCT116, HT29, and HT115), validated through phytochemical analysis and MTT assay. The extract exhibited dose-dependent cytotoxicity, with HT29 cells showing the highest sensitivity, followed by HCT116 and HT115 cells. The presence of bioactive compounds such as flavonoids, phenolics, alkaloids, and tannins contributes to the antioxidant and anti-cancer properties, potentially through mechanisms involving free radical scavenging, apoptosis induction, and inhibition of cell proliferation. The lower CTC50 values observed in HT29 cells highlight the extract’s potent inhibitory effects. Compared to conventional chemotherapeutic agents, the extract offers a natural and potentially safer alternative for cancer treatment. While the in vitro results are promising, further in vivo studies and clinical trials are necessary to confirm the therapeutic efficacy, optimal dosage, and molecular pathways involved in its anti-cancer activity.

**Authors’ Contributions**

GK conducted the experiments and performed data analysis. MB contributed to data interpretation and manuscript drafting. GK assisted in data analysis and provided essential materials. MB was responsible for experimental design, supervision, data analysis, manuscript writing, and revisions. All authors have reviewed and approved the final version of the manuscript.

**Disclosure Statement**

The authors declare that there are no conflicts of interest related to this study.

**Ethics Approval and Consent to Participate**

This study was reviewed and approved by the Ethics Committee under Study Number TRS/PT/023/039. The research was conducted at Trans-Genica Services Pvt. Ltd., Jalgaon, Maharashtra, India.

**Data Availability Statement**

All data generated or analyzed in this study are presented within this published article.

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