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Preferential Cytotoxic Effect of *Vaccinium* sect. *Cyanococcus* Fruit Extract in Human Lung Cancer Cells Related to Normal cells

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Abstract

The study aimed to assess the preferential cytotoxicity of the *Vaccinium* sect. *Cyanococcus* fruit extract (VCFE) in human lung cancer cells (A549) related to human normal/healthy kidney cells (HEK-293). VCFE was extracted using ethanol following the cold maceration technique. A qualitative phytochemical profile showed that VCFE was rich in phenolics and flavonoids. A quantitative phytochemical profile showed that one g of VCFE was found to contain 26.31 ± 1.09 mg gallic acid equivalent, GAE, 37.10 ± 1.82 mg quercetin equivalent, and 6.27 ± 0.39 mg tannic acid equivalent of total phenolics, total flavonoids, and total tannins, respectively. The EC_{50} value (effective concentration required to inhibit 50% of free radicals) of VCFE in ABTS free radical, DPPH free radical, and FRAP antioxidant assays was determined as 372.81 ± 20.58, 414.63 ± 33.08, and 504.58 ± 27.76 µg/mL, respectively. The IC_{50} value (concentration required to inhibit 50% of cell viability) of VCFE in human lung cancer cells (A549) and human normal kidney cells (HEK-293) was determined as 262.41 ± 23.03 and 608.05 ± 37.92 µg/mL, respectively. The findings revealed that the concentration of VCFE needed to inhibit the growth of A549 cancer cells was substantially lower than that needed to inhibit the growth of HEK-293 normal cells, demonstrating VCFE's selective cytotoxicity for cancer cells. The study concluded that VCFE might be effective in developing specific cancer-fighting medications.

Keywords: *Vaccinium* sect. *Cyanococcus*, antioxidant activity, cytotoxicity, anticancer activity, A549 cells, HEK-293 cells.

Introduction

The health benefits of fruits and vegetables are widely marketed. According to the 2010 Dietary Guidelines for Americans, 'you should have half fruits and vegetables on your plate'. A wide range of plant foods, including fruits and vegetables, provide varying amounts of nutrients and energy. Fruits and vegetables also include dietary fiber, which is associated with a decreased risk of obesity and cardiovascular disease. In addition to providing vitamins and minerals, fruits and vegetables are also good providers of phytochemicals, which have anti-inflammatory, phytoestrogenic, and antioxidant properties (1).

Among the different accessible fruits, small berry fruits are one of the richest sources of natural antioxidants and are consumed for their appealing color and distinctive flavor. Some chronic and degenerative diseases may be prevented by consuming them A widespread and widely grown genus of perennial flowering plants, blueberries produce blue or purple

berries. They belong to the *Vaccinium* genus and are included in the *Cyanococcus* section. Cranberries, bilberries, huckleberries, and blueberries from Madeira are also part of the Vaccinium family. Both wild (lowbush) and cultivated (highbush) commercial blueberries are indigenous to North America (2).

In ripe blueberries, anthocyanin flavonoids make up to 60% of polyphenols. Anthocyanins so likely contribute most to the health advantages of blueberries. Flavonoid and non-flavonoid polyphenolic chemicals are both present in blueberries. Proanthocyanidins and flavonols are two other types of flavonoids that can be present in blueberries. The hydroxycinnamic acid esters are prevalent non-flavonoid polyphenolic chemicals found in blueberries, especially contain chlorogenic acid. They impart powerful antioxidant capabilities that have a positive impact on health and provide blueberries with their reputation as functional foods. As worries about the potential negative effects of synthetic dyes have grown, the food sector also looked into the potential of anthocyanins as natural colors. Using a derivatization procedure that polymerizes anthocyanins and yields stable colored by-products can increase the stability of anthocyanins in food and drink. Using an improved methodology boosts both color intensity and color variability. Regular, moderate consumption of blueberries and/or anthocyanins has been linked by epidemiological research to better weight management, neuroprotection, and a lower risk of cardiovascular disease, and type 2 diabetes. Human clinical investigations using biomarkers provide data in favor of these conclusions. Blueberries' anti-inflammatory and antioxidant properties, as well as their advantageous effects on vascular and glucoregulatory function, are some of their more significant health benefits. The phytochemicals in blueberries may influence the gut microbiota and improve host health. These factors affect both the aging process and degenerative diseases and disorders (3,4,5).

In the present study, ethanolic extract of *Vaccinium* sect. *Cyanococcus* fruits were obtained and carried out the qualitative and quantitative phytochemical profiles. Following, the antioxidant activity of *Vaccinium* sect. *Cyanococcus* fruit extract (VCFE) was done by DPPH free radical, ABTS free radical, and FRAP assays. The preferential cytotoxic effect of VCFE was revealed in human lung cancer A549 cells related to human kidney HEK-293 cells by MTT assay and bright-field microscopic observation.

Materials and methods

Chemicals and reagents: Dulbecco's modified Eagle's medium (DMEM), trypsin, 0.22 µm syringe filter, amphotericin B, penicillin, streptomycin, Dulbecco's phosphate-buffered saline (DPBS), water (HPLC grade), and Whatman no.1 filter papers were procured from Sigma-Aldrich, Bengaluru, India. Fetal bovine serum (FBS) and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were supplied by HiMedia, Mumbai, India. From Tarsons, Bengaluru, India, Pvt Company the fundamental and specific grade of cell culture and all other assay required plasticware were received. The glassware was obtained from Borosil Company, Mumbai, India. These materials were water-logged with 5% of hypochlorite solution, finally washed with gentle soap and rinsed in water, at last dehydrated in an incubator (hot air oven inbuilt) at 70 °C. Ethanol (99.99%), methyl alcohol, potassium ferricyanide acetonitrile, trichloroacetic acid, dimethyl sulfoxide (DMSO), and other chemicals of satisfactory/fine ranking were attained as of Merck Millipore Corporation, Bengaluru, India.

Collection and preparation of fruit extract: Fruits from *Vaccinium* sect. *Cyanococcus* were purchased from a nearby market in Vijayawada, Andhra Pradesh. To remove dust and dirt, the fruits were rinsed repeatedly with clean double distilled water. The fruit materials were then evenly dispersed and dried. At last, the dried samples were processed in an electrical

chopper to acquire fine powder for future use. Then, using the cold maceration method, 10 g of *Vaccinium* sect. *Cyanococcus* (VC) fruit extract powder was dissolved in 100 mL of ethanol. After three days of maceration, the filtrate was filtered through Whatman® grade 1 filter paper and lyophilized at -40 °C to concentrate. The obtained VC fruit extract (VCFE) powder was used for additional research after being stored at 4° C in a sterile airtight bag.

Qualitative phytochemical analysis of VCFE: The VCFE powder was subjected to a qualitative phytochemical examination utilizing established techniques to determine the presence of active chemical components (6,7). About 10 mg of VCFE powder was suspended in 10 mL of ethanol and utilized for qualitative analysis.

Alkaloids - Mayer's test: Briefly, 4 mL of 1% hydrochloric acid was added to 2 mL of ethanolic VCFE, which was then warmed and filtered to determine the presence of alkaloids. Six drops of Mayor's/Dragendroff reagent were applied individually to 1 mL of ethanolic VCFE. The presence of respective alkaloids was seen by a creamish/orange precipitate.

Steroids - Lieberman-Buchard's test: Briefly, 1 mL of ethanolic VCFE was treated with 0.5 mL acetic acid, chloroform, and 1 mL of concentrated sulfuric acid. The occurrence of steroids was shown by the formation of a reddish brown ring at the separating level of the two liquids.

Flavonoids - sodium hydroxide test: Briefly, 3 mL of ethanolic VCFE was pipetted out, 10 mL of distilled water was added, and the mixture was stirred. Following, 1 mL of 10% sodium hydroxide was also added to the mixture. The presence of flavonoids was revealed by a yellow coloring.

Tannins - ferric chloride test: Briefly, in a test tube, 1 mL of the ethanolic VCFE was taken in 10 mL of distilled water and heated. Following, drops of ferric chloride (10%) were added to it. The green color was visible in the solution.

Anthraquinones: Bourn stranger's test: Briefly, 5 mL of a 10% ammonia solution was added to 2 mL of ethanolic VCFE. The presence of anthraquinones is indicated by the production of red color or precipitation.

Reducing sugar - Free reducing sugar test: Briefly, 2 mL of ethanolic VCFE was added to 5 mL of Fehling solutions in a test tube and heated in an 80 °C water bath for 10 min. The presence of reducing compounds was determined by the development of a brick red precipitate or solution.

Carbohydrates - Molisch's test: Briefly, 2 drops of Molisch's reagent were administered to 2 mL of ethanolic VCFE and shaken vigorously, followed by 2 mL of concentrated sulfuric acid on the test tube's sides. The presence of carbohydrates was instantly noticed by the formation of a reddish violet ring at the intersection of two layers.

Phenolics - Lead acetate test: Briefly, to 2 ml of ethanolic VCFE, 5 mL of 10% w/v lead acetate was added. The presence of phenolics is defined by the presence of white precipitate.

Flavonoids - Aluminum chloride test: Briefly, 3 mL of ethanolic VCFE was combined with 4 mL of 1% aluminum chloride in methanol in a test tube. The presence of flavonoids was revealed by the formation of a yellow color.

Terpenoids - Salkowski's test: Briefly, in a test tube, 5 mL of ethanolic VCFE was added with 2 mL of chloroform. To produce a layer, 3 mL of concentrated sulfuric acid was added to the mixture slowly. Once terpenoids constituents were present, an interface with a reddish-brown color resulted.

Amino acids - Ninhydrin test: Briefly, in a test tube, a few drops of ninhydrin reagent were administered to ethanolic VCFE solution (1 mL). The presence of amino acids was indicated by the appearance of a purple color.

Proteins - Biuret test: Briefly, 2 mL of ethanolic VCFE was mixed with 1 mL of 40%

sodium hydroxide solution and 1-2 drops of 1% copper sulfate solution (Biuret reagent). The occurrence of peptide linkage was shown by a violet coloring.

Glycosides - Keller – Kiliani test: Briefly, 2 mL of glacial acetic acid and 0.5 mL of 1% ferric chloride were added to 3 mL of ethanolic VCFE to identify cardiac glycosides. Then 1 mL of strong sulfuric acid was applied to it. The presence of cardiac glycosides was suggested by the greenish-blue color.

Quantitative phytochemical analysis of VCFE:

Quantification of total phenolics: According to the method of Nagaraj & Samiappan, the total concentrations of phenolic compounds present in VCFE were quantified and the analysis was carried out with minor modifications (8). About 0.10 to 1 mL of extract was transported to a test tube, the final volume was adjusted to 2 mL by adding distilled water, and then 1 mL of Folin-Ciocalteu reagent was added and allowed to stand for 3 min before adding 3 mL of sodium carbonate (2%). After that, the solution was stirred for 2 hrs at room temperature on a shaker, and the absorbance was recorded at 760 nm. For the calibration curve, catechol was employed as the standard. The phenolic component content was calculated using a linear equation based on the calibration curve and the findings were represented as mg gallic acid equivalents/g of VCFE (GAE mg/g).

Quantification of total flavonoids: The total flavonoid content was measured using the Kalagatur et al. procedure with minor modifications (9). In a brief, 0.15 ml of 10% aluminum chloride in methanol was added with the 0.25 – 1 mL of VCFE, and the tubes equalized with 1.25 mL of distilled water. In addition to that 75 µL of 5% sodium nitrite solution was incorporated and after a few minutes, 0.5 mL of 0.1M sodium hydroxide was added and the final volume 2.5 mL was made with distilled water. At 415 nm, absorbance was determined. The quantities of flavonoid components were estimated using the calibration curve equation derived from the standard quercetin graph, with the result expressed as quercetin equivalents mg/g (mg QE/g).

Quantification of total tannins: T he tannin quantification was done using the Gunti al. technique with minor modifications (10). About 6 mL of 8 mM potassium ferric cyanide and 0.1M ferric chloride were added to 6 mL of 0.1N hydrochloric acid, respectively. After mixing the components (6 ml) with 4 mL of VCFE, the mixture was maintained at an ambient temperature for 20 min. Finally, the optical density was then measured at 700 nm using a multimode reader. As a control, tannic acid was utilized. The tannin content of VCFE was measured and results were denoted as milligram of tannic acid equivalents per gram of extract (mg TAE/g).

Antioxidant activity of VCFE:

DPPH free radical scavenging assay: The antioxidant potential of VCFE was done as per the methodology of George et al. with minor modifications (11). Briefly, different concentrations of VCFE and 50 µL of 0.1 mM DPPH in methanol were combined to form a 100 µL reaction mixture. The reaction mixtures were then fully combined, incubated for 30 min at 37 °C with 150 rpm shaking and absorbance was recorded using a microplate reader at 517 nm. The negative control was 50 µL of methanol and 50 µL of 0.1 mM DPPH in methanol. The reference control was a mixture of 50 µL of butylated hydroxyl toluene (BHT) at various concentrations (20-100 µg/mL) and 50 µL of 0.1 mM DPPH in methanol. The outcome of the study was expressed as an EC_{50} value, which means an effective concentration of VCFE is required to decrease the absorbance of DPPH radicals by 50%. The radical scavenging effect of VCFE as a percentage was estimated as follows:

Dpph radical scavenging = $Control_{abs}$ – $Treatment_{abs}$ / $Treatment_{abs}$ x 100

Where, $control_{abs}$ is the control's absorbance and $treatment_{abs}$ is the treatment's absorbance.

ABTS free radical scavenging assay: The ability of VCFE to scavenge ABTS free radicals was assessed following the technique of Kumar et al. and Rosaiah et al. with minor modifications (12,13). A stock solution of 7.4 mM ABTS and 2.6 mM potassium persulfate was prepared, mixed in an equal volume, and kept in the dark for 12 to 16 hrs to make the ABTS working solution. For the experiment, 135 µL of ABTS working solution was mixed with 15 µL (different concentrations) of VCFE, and the mixture was then kept in the dark for two hrs. The solution's absorbance was determined at 750 nm. For the negative control, 135 µL of the ABTS working solution was combined with 15 µL of ethanol. The reference control was created by adding varying doses of BHT (20-100 µg/mL) to 135 µL of the ABTS working solution. The outcome of the study was expressed as an EC_{50} value, which means an effective concentration of VCFE is required to decrease the absorbance of ABTS radicals by 50%. The radical scavenging effect of VCFE as a percentage was estimated as follows:

Dpph radical scavenging = $Control_{abs}$ – $Treatment_{abs}$ / $Treatment_{abs}$ x 100

Where, $control_{abs}$ is the control's absorbance and $treatment_{abs}$ is the treatment's absorbance.

FRAP assay: The Sudha et al. approach with minor modifications has been used to evaluate the reduction power (14). Briefly, variable concentrations of VCFE were quickly prepared, and 0.1 mL of each were then mixed with sodium phosphate buffer (pH 6.6 & 0.2 M) and potassium ferricyanide (1 g/100 mL water), which has a 1% concentration. The mixture was then immediately stored for quick cooling after being heated to 50 °C for 20 min. Afterward heating, trichloroacetic acid (10% w/v) was added to stop the reaction, and then the mixture was centrifuged for 10 min at 3000 rpm. The supernatant was mixed with 0.1% ferric chloride and its absorbance was determined spectrophotometrically at 700 nm. The outcomes were contrasted with those from BHT, which served as a standard control. The study's findings were expressed as an EC50 value, which is the effective amount of VCFE (an antioxidant) needed to reduce absorbance (deep blue hue) during ferricyanide conversion to ferrocyanide by 50%.

Cytotoxic effect of VCFE: The National Centre for Cell Science (NCCS) in Pune, India provided the human lung cancer cell lines A549 and human normal kidney HEK-293 cell line. These cells were cultured as adherent cultures at 37 °C in DMEM medium supplemented with 10% FBS, 2 mM L-glutamine, 100 units/mL of penicillin, and 0.1 mg/mL of streptomycin in a humid environment that typically contains 5% CO_2. When cells reached 80% confluence, they were trypsinized (phosphate buffer saline containing 0.25% trypsin and 0.1% EDTA), collected by centrifugation at 1,500 rpm for 5 min (REMI, India), and suspended in DMEM medium without FBS used for further studies.

MTT assay: The cytotoxic effect of VCFE on A549 cancer cells and HEK-293 normal cells was revealed by MTT assay as per the methodology of Swaminathan et al. with minor modifications (15). Briefly, 1.5×10^4 cells were seeded in 96-well plates in FBS-free DMEM medium and allowed to adhere for 8 hrs. Following, different concentrations of VCFE were treated to cells for 24 hrs. Next, media was replenished with 50 µL of MTT reagent (5 mg/mL in phosphate buffer saline pH 7.4) and incubated for 4 hrs in dark. Following this, MTT reagent was replaced with DMSO to dissolve the formazan crystals. Cisplatin was used as a standard anticancer agent. The cell viability was calculated with respect to control (100%). The results were expressed as IC_{50} value, which means concertation of VCFE is required to inhibit 50% of cell viability.

Micro-morphological observation: Briefly, 50,000 cells were seeded in cell culture dishes in a DMEM medium, which was free from FBS

and allowed to adhere for 8 hrs. Following, cells were treated with VCFE for 24 hrs. The images of the cells were captured using an inverted microscope (Olympus, Japan) and micro-morphology of cells was considered to evaluate the inhibitory activity of VCFE (16). The cisplatin was used as a standard anticancer agent.

Statistical analysis: The assays were carried out in triplicates (n = 3) and obtained results were expressed as mean ± standard deviation. The obtained data were analyzed by one-way ANOVA and statistical analysis was done by student's t-test.

3. Results and discussion

Qualitative phytochemical analysis of VCFE: Most of the plant extracts are available in their crude form. The desired plant extracts can be obtained via a variety of extraction techniques. Soxhlet and cold maceration are the two most popular extraction techniques. Soxhlet extraction has been practiced for many years, however, it takes a lot of time and uses a lot of solvents. Additionally, Soxhlet extractor unique devices are required for the procedure. Whereas, cold maceration, is a simple process that doesn't demand for any specialized equipment. Due to the low extraction temperature, which is similar to cold pressing, cold maceration always produces an aroma that is identical to that of the original plant material without degrading the thermolabile chemicals contained in the fractions (17). Therefore, in the present study, the cold maceration technique was chosen oven Soxhlet extraction technique to obtain VCFE.

Various assays were used in the study to disclose the qualitative phytochemical profile of VCFE, and the results are displayed in **Table 1**. The results showed that VCFE was rich in phenolics and flavonoids. In support of our study, previous reports suggested that blueberries are rich in polyphenols and anthocyanin flavonoids make up to 60% of polyphenols (3). Also, in our study, VCFE was found to contain moderate levels of carbohydrates, alkaloids, terpenoids, and proteins.

Table 1: Qualitative phytochemical profile of VCFE.

Phytochemical component	Level of attendance
Carbohydrates	++
Reducing sugars	++
Proteins	++
Amino acids	++
Alkaloids	++
Saponins	+
Tannins	+
Steroids	-
Flavonoids	+++
Phenols	+++
Glycosides	+
Terpenoids	++
Anthraquinones	-

High = +++; Moderate = ++; Low = +; Absent = -

Quantitative phytochemical analysis of VCFE: Quantitative phytochemical analysis of VCFE was revealed by determining the total phenolics, total flavonoids, and total tannin content and they were determined by Folin-Ciocalteu, aluminum chloride, and ferric chloride methods, respectively. The results showed that VCFE was high phenolics and flavonoid content. The total tannin content was lower compared to total phenolic and total flavonoid content (**Fig. 1**). The quantity of total phenolics, total flavonoids, and total tannins in one g of VCFE was determined to be 26.31 ± 1.09 mg GAE, 37.10 ± 1.82 mg QE, and 6.27 ± 0.39 mg TAE, respectively. When the concentration of the VCFE was raised, total phenolics, total flavonoids, and total tannins continued to increase in the amount and showed a dose-dependent pattern (**Fig. 1**).

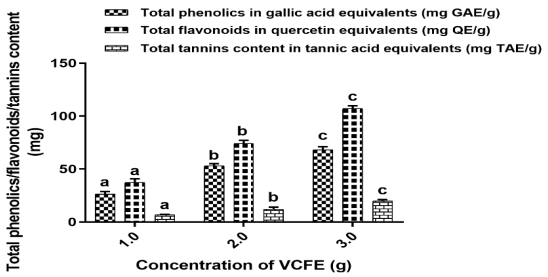

Figure 1: Quantitative phytochemical profile

of *Vaccinium* sect. *Cyanococcus* fruit extract (VCFE). The analysis was carried out in triplicates (n = 3) and the results were expressed as mean ± standard deviation. The statistical significance between the test samples within the respective analysis was determined by Tukey's test and a *p*-value ≤ 0.05 was considered significant. The bars in the particular study with different alphabetic denote the statistically significant (*p*-value ≤ 0.05).

Both phenolic compounds and flavonoids are well-known as antioxidants and have attracted researchers for a long time due to their advantages for human health and their potential to treat and prevent a wide range of disorders. Epidemiological research revealed an inverse correlation between the consumption of foods high in polyphenols and the risk of developing chronic human diseases. The favorable disruption in oxidative chain reactions inside cells is caused by the production of phenoxyl radicals as a result of the phenolic groups of polyphenols accepting an electron. According to researchers, the reducing polyphenols found in food and drink showed an increase in plasma antioxidant activity because they accumulated in plasma along with endogenous antioxidants, which in turn help with iron absorption as a pro-oxidative dietary component. Consuming a diet high in polyphenols works as an antioxidant and guards DNA against oxidative damage. In addition to preventing oxidative damage to cells and their constituent parts, polyphenols also lower the risk of oxidative stress, which is associated with a number of degenerative disorders (18,19,20). The study shows that VCFE is rich in polyphenols and therefore, perfectly apt for various applications in biomedicine.

Antioxidant activity of VCFE: Antioxidants are molecules that may shield cells from free radicals, which have been linked to cancer, heart disease, and other illnesses. In the present study, the antioxidant potential of VCFE was determined by ABTS free radical, DPPH free radical, and FRAP assays. The EC_{50} value (effective concentration required to inhibit 50% of free radicals) of VCFE in ABTS free radical, DPPH free radical, and FRAP assays was determined as 372.81 ± 20.58, 414.63 ± 33.08, and 504.58 ± 27.76 µg/mL, respectively. The VCFE has exhibited dose-dependent radical scavenging potential in all evaluated methods (**Fig. 2**). The obtained results were in accordance with the qualitative and quantitative phytochemical analysis of VCFE. The study concludes that VCFE-rich antioxidant potential could be highly useful in scavenging free radicals and help in relieving oxidative stress-mediated diseases like cancer, heart illness, Parkinson's and other neuro disorders, etc.

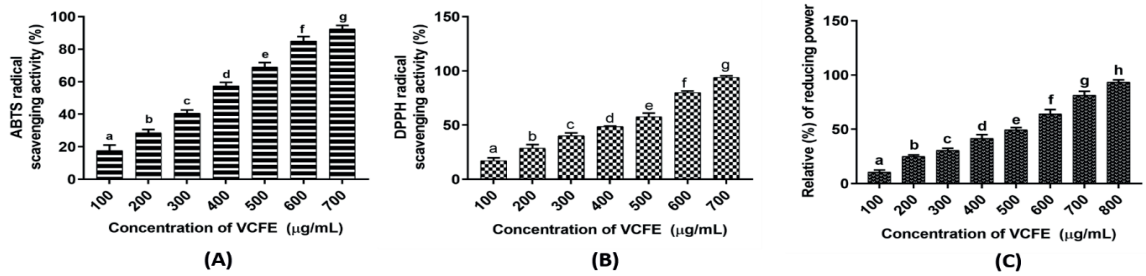

Figure 2: Dose-dependent antioxidant potential of VCFE determined by (A) ABTS, (B) DPPH, and (C) FRAP free radical scavenging assays. The analysis was carried out in triplicates (n = 3) and the results were expressed as mean ± standard deviation. The statistical significance between the test samples within the respective analysis was determined by Tukey's test and a *p*-value ≤ 0.05 was considered significant. The bars in the particular study with different alphabetic denote the statistically significant (*p*-value ≤ 0.05).

Cytotoxic effect of VCFE: Using the MTT assay and micro-morphological studies, it was determined that VCFE has a selective cytotoxic impact on human lung cancer cells (A549) compared to human normal kidney cells (HEK-293). The study found that VCFE has dose-dependently showed the cytotoxicity (growth inhibition) towards human lung cancer cells (A549) (**Fig. 3**). The IC_{50} value (concentration required to inhibit 50% of cell viability) of VCFE in A549 cancer cells and HEK-293 normal cells was determined as 262.41 ± 23.03 and 608.05 ± 37.92 µg/mL, respectively. The IC_{50} value of cisplatin in A549 and HEK-293 cells were noticed as 5.81 ± 0.03 and 14.10 ± 0.29 µM, respectively. The results showed that the concentration of VCFE required to inhibit the growth of A549 cancer cells was much lower compared to normal HEK-293 cells, which shows the preferential cytotoxicity of VCFE towards cancer cells.

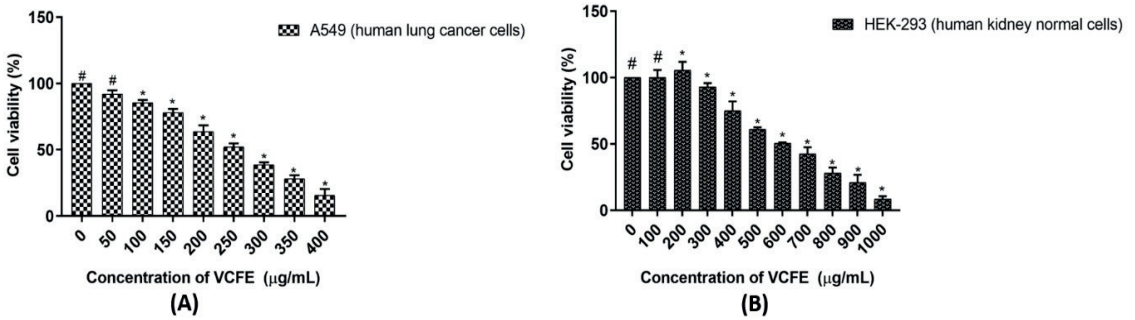

Figure 3: Cytotoxic effect of VCFE on (A) human lung cancer A549 cells and (B) human normal kidney HEK-293 cells. The analysis was carried out in triplicates (n = 3) and the results were expressed as mean ± standard deviation. The statistical significance between the control and test samples within the respective analysis was determined by Dunnett's test and a *p*-value ≤ 0.05 was considered significant. The bars in the particular study with different alphabetic denote the statistically significant (*p*-value ≤ 0.05).

When compared to untreated cells (control), the IC_{50} value of VCFE and cisplatin was found to have an impact on the micro-morphology of cancer and normal cells. Control cells showed a healthy shape in both cancer and normal cells (**Fig. 4**). The micro-morphology of cells treated with VCFE and cisplatin at IC_{50} values showed adverse alterations, including loss of cell shape and structure, evidence of cellular debris, development of apoptotic bodies, etc.

The VCFE may be acted as a pro-oxidant in cancer cells and inhibited the growth of cancer cells. As opposed to this, VCFE has increased the proliferation of human normal kidney cells (HEK-293) up to a concentration of 200 µg/mL, demonstrating that it may function as an antioxidant and shield the normal cells from free radicals.

Pro-oxidants, commonly referred to as "oxidation therapy," have gained popularity as a cancer prevention method in recent years. It has been found that using medications and nutritional supplements that produce hydrogen peroxide, such as paclitaxel, resveratrol, EGCG, and curcumin, can effectively kill tumor cells while barely affecting normal cells. Indeed, it has been demonstrated that several chemotherapeutic drugs frequently used in clinical practice, including paclitaxel, cisplatin, doxorubicin, arsenic trioxide, and etoposide, contribute to the formation of ROS and that this effect is mediated by the generation of H_2O_2, in part through NADPH oxidase activation (21,22). In accordance with these reports, the

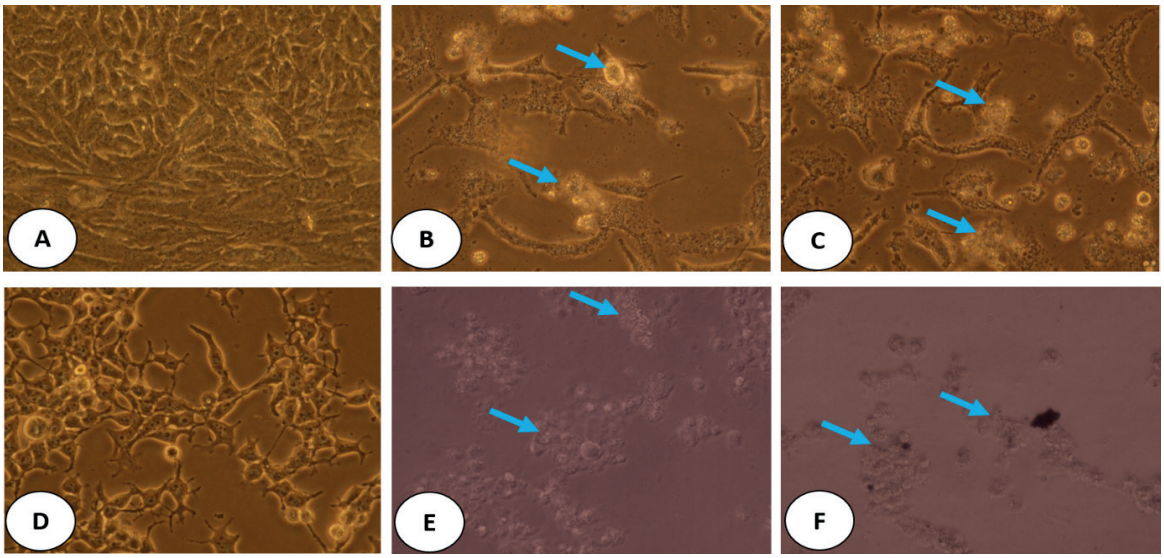

Figure 4: Micro-morphology of cancer (A549) and normal (HEK-293) cells treated with IC_{50} value of VCFE and cisplatin. The images were captured at 400x. (A) A549 control cells. (B) A549 cells treated with IC_{50} value of VCFE. (C) A549 cells treated with IC_{50} value of cisplatin. (D) HEK-293 control cells. (E) HEK-293 cells treated with IC_{50} value of VCFE. (F) HEK-293 cells treated with IC_{50} of cisplatin.

present study concluded that VCFE could be highly useful in developing discriminating drugs against cancer.

4. Conclusion

VCFE was successfully extracted by the cold maceration technique. Qualitative and quantitative analysis showed that VCFE was rich in polyphenols. VCFE has exhibited potent antioxidant activity in DPPH free radical, ABTS free radical, and FRAP assays, and showed that VCFE could be potentially useful in treating oxidative-stress mediated diseases. In cytotoxic analysis, findings revealed that the concentration of VCFE needed to inhibit the growth of A549 cancer cells was substantially lower than that needed to inhibit the growth of HEK-293 normal cells, demonstrating VCFE's selective cytotoxicity for cancer cells. The study concluded that VCFE could be potentially useful in developing discriminating drugs against cancer.

Conflict of interest

There is no conflict of interest

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