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Research Article

Phytochemical, Antioxidant and Anticancer Properties of Ayusip (*Vaccinium uliginosum L.*) from Benguet Province, Philippines

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Abstract
Traditional medicinal knowledge remains an important source of healthcare for many communities in the Philippines, particularly in rural and Indigenous areas where access to modern treatments is limited. In this context, *Vaccinium uliginosum* (locally known as Ayusip), a plant used by indigenous peoples in Tublay, Benguet Province, has drawn interest due to its reported therapeutic applications for ailments such as cough, flu, and even cancer. This study investigates the cytotoxic properties of Ayusip, aiming to provide scientific validation for its traditional use and assess its potential as a source of bioactive compounds for cancer treatment. Leaves of *V. uliginosum* were collected, identified, and subjected to methanol extraction, with further partitioning using *n*-hexane and ethyl acetate. Phytochemical analysis indicated high levels of flavonoids, saponins, steroids, and tannins. Quantification showed significant phenolic (24.58±8.11 GAE mg/g) and flavonoid (121.38±14.04 QE mg/g) content. Antioxidant testing (DPPH assay) revealed strong activity (97.86±1.33%) at 25 mg/mL. The cytotoxicity of *V. uliginosum* was assessed against normal embryonic lung fibroblasts (MRC-5) and cancer cell lines HCT-116 (colorectal) and A549 (lung adenocarcinoma) using the MTT assay. Results showed that only the *n*-hexane extract was toxic to MRC-5 cells at high concentrations, while methanol and *n*-hexane extracts were highly cytotoxic to HCT-116 cells. In A549 cells, only *n*-hexane extract exhibited cytotoxicity. These findings suggest that *V. uliginosum* has promising cytotoxic effects, particularly at higher concentrations, with notable activity against HCT-116 cells. Interestingly, its cytotoxicity is biphasic: high concentrations induce maximum toxicity, whereas lower concentrations may encourage cell proliferation. Overall, this study offers insights into the cytotoxic potential of *V. uliginosum*, a traditionally used plant among Benguet's IPs, highlighting its potential medicinal value.

Keywords: MTT assay, cytotoxicity, indigenous people, medicinal plant, antioxidative, Ayusip

1. INTRODUCTION

The Philippines, a lower-middle-income country in Southeast Asia with over 110 million people, faces a growing burden of cancer. Lung cancer remains the leading cause of cancer-related deaths, driven by high smoking rates with nearly 25% of Filipinos aged 15 and above smoke, and 80% of the world's 1.3 billion smokers live in LMICs like the Philippines [1][2]. Colorectal cancer is also on the rise, now ranking as the third most common cancer in the country [2][3]. The financial strain of conventional cancer treatments often makes them inaccessible for many Filipinos, prompting a reliance on more affordable alternatives such as traditional medicine. For a long time, traditional medicine has been essential to healthcare systems all throughout the world over 170 countries utilize traditional therapies including herbal medicine, acupuncture, and indigenous healing systems as part of their national health strategies [4]. While sometimes labeled as pre-scientific, traditional medicine has profoundly influenced modern pharmacology. Notably, approximately 40% of pharmaceuticals are derived from natural products or traditional knowledge, including critical drugs like aspirin, artemisinin, and certain childhood cancer treatments.

In countries like the Philippines, where local plant knowledge is still deeply embedded in community practices, such medicinal resources offer valuable, affordable alternatives for disease prevention and treatment. One such plant, *Vaccinium uliginosum,* locally known as Ayusip, is utilized by IPs in Tublay, Benguet Province,

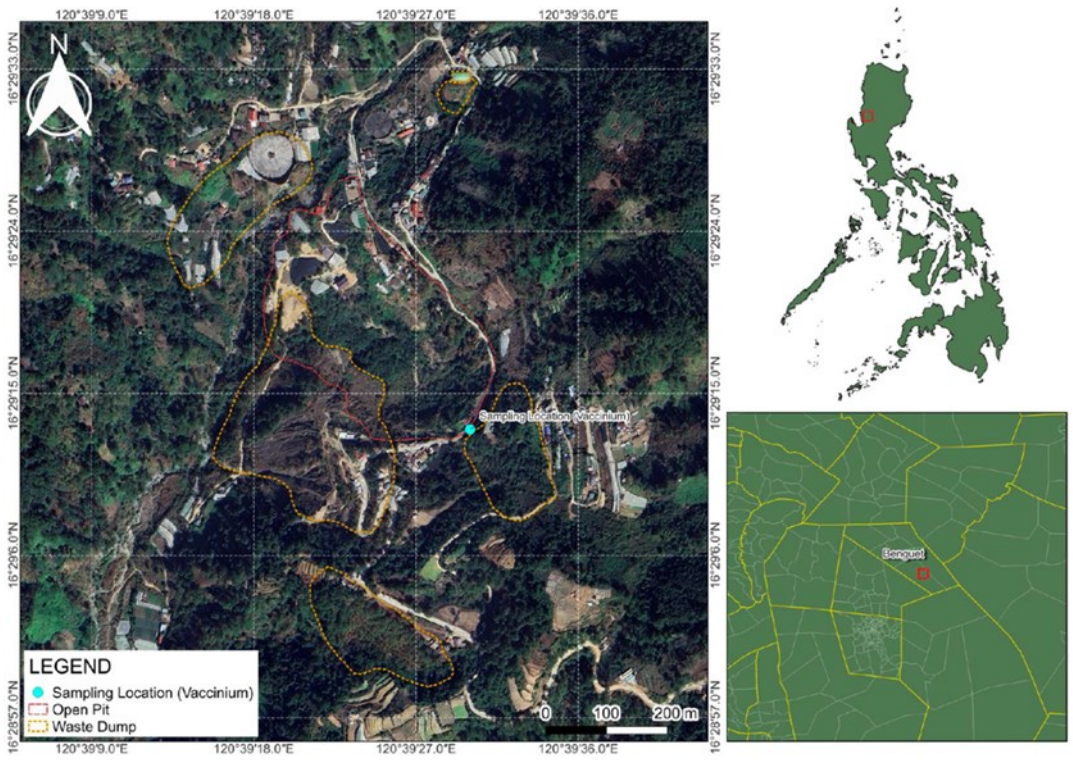

Figure 1. Sampling site for the collection of *Vaccinium uliginosum* utilized in this study, the exact sampling area is marked with blue dot.

Philippines [5]. Belonging to the Ericaceae family, *V. uliginosum*—commonly called bog bilberry or northern bilberry—is a deciduous shrub that typically grows between 0.3 to 2 m tall [6]. It is globally distributed, especially across the boreal and temperate zones of the Northern Hemisphere, including parts of Europe, Asia (notably Siberia, China, and Japan), and North America [7]-[9]. In the Philippines, it thrives in high-altitude, mountainous areas such as Ilocos Sur, Mountain Province, Ifugao, Benguet, Laguna-Quezon, Mindoro, and Mindanao, often favoring steep slopes and volcanic soils [10].

Medicinally, the plant has a long history of use in both traditional Asian and European herbal practices. Historically, its fruits, leaves, and stems have been used for treating conditions such as diabetes, poor vision, fever, infections, and inflammation, with the leaves and fruits being the most widely utilized for their therapeutic properties. In Tublay, the leaves and fruits are used to enhance eyesight, manage diabetes, and serve as antioxidant and anticancer remedies [10][11]. The Kalanguya tribe in Ifugao also uses a decoction of the plant stems as a fever remedy [11]. Ethnobotanical reports from other regions similarly highlight its use in managing gastrointestinal, inflammatory, and metabolic disorders.

Scientific investigations have begun to validate these traditional claims. A recent study on *V. uliginosum* revealed a rich and diverse phenolic profile in its leaves. Seventeen phenolic compounds were consistently identified across four extraction techniques—high-pressure extraction (HPE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and conventional ethanol extraction (CE). These compounds primarily belonged to hydroxycinnamic acids, flavanols, and flavonols. Chlorogenic acid, neochlorogenic acid, and caffeic acid were the most abundant phenolic acids, while quercetin-glucuronide was the dominant flavonol. Alternative extraction methods (HPE, UAE, MAE) yielded higher concentrations of these compounds, along with greater antioxidant and selective antimicrobial activity compared to conventional ethanol extraction [12]. These findings highlight the plant's potential for use in functional foods and

pharmaceuticals, and the importance of optimized extraction techniques to enhance its therapeutic benefits. Hence, this study investigates the bioactive compounds present using qualitative test, antioxidative using DPPH assay, and cytotoxic activities using MTT assay of *V. uliginosum* warranting further investigation for potentially safe plant-based therapy. To the best of our knowledge, this is the first study from the Philippines to scientifically confirm the medicinal properties of *V. uliginosum*, filling a critical gap in ethnobotanical research within the region.

2. MATERIALS AND METHODS

2.1. Plant Samples Collection and Authentication

V. uliginosum was collected from Sto. Niño, Tublay, Benguet Province, Philippines (Figure 1). Certification of Precondition (CPIKSP-CAR-2022-017) from the National Commission on Indigenous Peoples and a Gratuitous permit (DENR-CAR-08-2022) from the Department of Environment and Natural Resources-Baguio were obtained through the Bio+Mine project before collecting the plant samples (Figure 1) [5]. Documentation was done in the form of detailed field notes and photographs which recorded the habit, inflorescence, infructescence, leaf and stem morphology (Figure 2). The plant specimen was verified and identified by Michael Muhmin E. Manting, a Botanist from MSU-Iligan Institute of Technology. A voucher specimen was deposited at the University of Santo Tomas Herbarium (USTH 018576).

2.2. Preparation of the Methanolic Crude Leaf Extracts

Freshly harvested *V. uliginosum* leaves were air-dried for four weeks in the dark at room temperature and then finely ground into a powder. The powdered leaves of 100 g were soaked in 95% methanol for 3 days and subsequently filtered using Whatman filter paper. The resulting filtered methanolic extract was processed using a rotary evaporator to obtain a crude extract then oven-dried at 37 °C to completely dry off any remaining solvent, resulted in a powder form and stored in the refrigerator until used.

2.3. Preliminary Screening of Phytochemicals

The methanolic crude leaf extract of *V. uliginosum* underwent methods outlined in previous protocols [13][14]. The crude leaf extract of *V. uliginosum* was qualitatively evaluated for the presence of various phytochemicals including alkaloids, saponins, flavonoids, tannins, cyanogenic glycosides, steroids, and anthraquinones. Methanolic crude leaf extract phytochemical screening was analyzed in the Department of Chemistry, MSU-Iligan Institute of Technology. Detected bioactive compounds were categorized

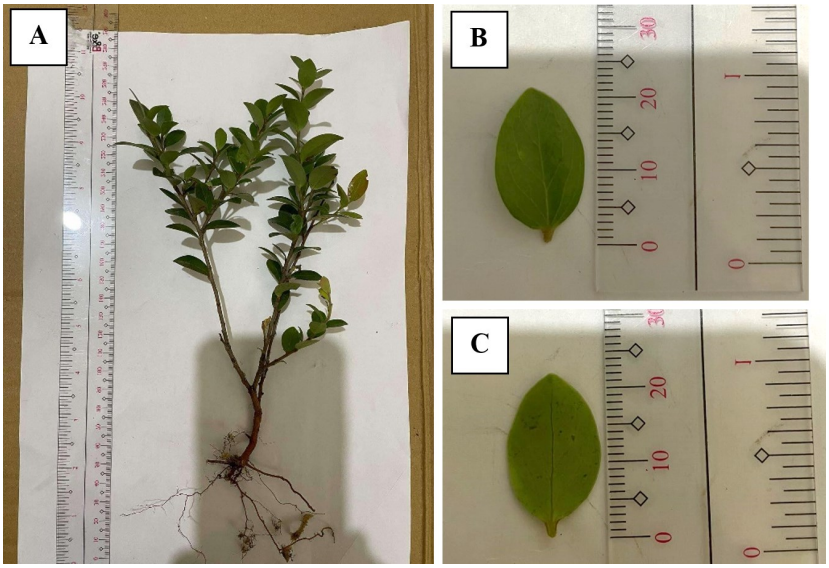

Figure 2. (A) Representative whole plant of *Vaccinium uliginosum* collected from Sto. Niño, Tublay, Benguet Province with the plant height as 280 cm. Representative leaf of *V. uliginosum* showing (B) front view, and (C) back view.

Table 1. Phytochemical screening of leaf crude extract of *Vaccinium uligonosum*.

Sample	Phytochemical screening of leaf crude extract of *V. uligonosum*						
	Alkaloids	Anthraquinones	Cyanogenic-Glycosides	Flavanoids	Saponins	Steroids	Tannins
Leaves	+	-	-	+++	+++	+++	+++

Note: high detection (+++), moderate detection (++), low detection (+), and no detection (-).

based on their abundance levels, where a single plus sign (+) indicated the presence of the active compound, while multiple plus signs denoted the degree of detection, with a greater number of plus signs indicating higher detection level (see Table 1).

2.4. Determination of Total Phenolic Content of the Methanolic Crude Leaf Extract of V. uliginosum

To determine the total phenolic content (TPC), we utilized an adjusted method using the Folin-Ciocalteu reagent [15]. Working solutions of 2, 1, 0.5, and 0.1 mg/mL were prepared by diluting the stock solution. In a 96-well plate, the *V. uliginosum* extract was seeded at concentrations A (0.1 mg/mL), B (0.5 mg/mL), C (1 mg/mL), and D (2 mg/mL). Methanol (100 µL) was added to each well, followed by 50 µL of diluted Folin-Ciocalteu reagent for rows A–D, and 25 µL of methanol for rows E–H. After incubating for 5–10 min, 25 µL of 0.35M Na_2CO_3 was added to rows A–D, and 25 µL of methanol to rows E–H, with further incubation for 5–10 min. Absorbance was measured at 750 nm, and results were calculated based on the gallic acid calibration curve ($y = 5.0472x$, $R^2 = 0.4707$).

2.5. Determination of Total Flavonoid Content of the Methanolic Crude Leaf Extract of V. uliginosum

The total flavonoid content (TFC) of the methanolic extract from *V. uliginosum* was determined using the $AlCl_3$ colorimetric method [16]. The extract was initially diluted to 10 mg/mL and further prepared at concentrations of 2, 1, 0.5, and 0.1 mg/mL. In a 96-well plate, 100 µL of methanol was added to each well, followed by 25 µL of 10% w/v $AlCl_3$ for rows A–D and 25 µL of methanol for rows E-H. After 5–10 min of incubation, 25 µL of 1M $NaNO_2$ solution was added to rows A–D, while 25 µL of methanol was added to rows E–H. Absorbance was measured at 520 nm, and TFC was calculated using the quercetin calibration curve ($y = 1.2862x$, $R^2 = 0.9984$).

2.6. DPPH Radical Scavenging Assay of Methanolic Crude Leaf Extract of V. uliginosum

The evaluation of the anti-radical properties of the *V. uliginosum* methanolic extract was conducted using a 2,2-diphenyl-1-picrylhydrazyl (DPPH)

assay following previous protocol with modifications [17]. Briefly, 5 mg of the *V. uliginosum* crude methanolic extract was dissolved in 5mL of methanol and subjected to serial dilution to get the following two-fold concentrations respectively (200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 ppm). Then a 100 µL of 0.2 mM DPPH solution was added to each well on the plate. The seeded plate was then incubated for 30 minutes before measuring the absorbance using a microplate reader at 517 nm using a PerkinElmer VICTOR X3 Multi-Mode Plate Reader (PerkinElmer, Inc., USA). The percentage of DPPH radical scavenging was calculated using the following Equation (1).

$$\text{Scavenging of DPPH (\%)} = \frac{[A_0 - A_1]}{A_0} \; x \; 100\% \qquad \textbf{(1)}$$

where, A_0 is absorbance of the blank solution and A_1 is absorbance of the *V. uliginosum* extract sample. The efficient concentration (EC_{50}) value is the concentration required to obtain a 50% antioxidant effect and was determined using GraphPad Prism v. 10.2.3. All measurements were conducted in triplicates to ensure accuracy and reliability. The results were compared against established ranges for antioxidant activity: EC_{50} of 10–50 µg/mL indicate strong antioxidant activity; 50–100 µg/mL indicate intermediate antioxidant activity; and >100 µg/mL indicate weak antioxidant activity [18].

2.7. Solvent Partitioning

Briefly, 5 g of crude leaf extract was dissolved in 100 mL methanol. Using a separatory funnel, the polar plant sample extract was decanted, and an equal volume of *n*-hexane solvent was added. The solution was carefully mixed thoroughly and allowed to separate for about 1 h. The upper (*n*-hexane) and lower (methanol) layers were then collected separately. The retained methanol layer was diluted with an equal volume of distilled water and subsequently subjected to a second partitioning step using an equal volume of ethyl acetate, following the same procedure. Each collected fraction (*n*-hexane, ethyl acetate, and methanol) was concentrated using rotary evaporation and then dried overnight in an oven and stored in the refrigerator for further analysis.

2.8. Cell Lines and Culture

Cell cultures of MRC-5 (normal human lung fibroblast cell line), HCT 116 (human colon cancer cell line), and A549 (human lung cancer cell line) were purchased from the American Tissue Culture Collection (ATCC, Rockville, MD). All cell-based assays were done at the Mammalian Cell Culture Service Laboratory (MCCL) at the University of the Philippines, Diliman. Briefly, cells were grown at 37 °C in a humidified environment with 5% CO_2. MRC-5 cells were cultured in ATCC-formulated Eagle's minimum essential medium (ATCC, Rockville, MD, Catalog No. 30-2003) supplemented with 10% not heat-inactivated fetal bovine serum (Gibco™ Thermo Fisher Scientific Inc., USA, Catalog No. 10270-106) and 1% antibiotic-antimycotic solution (100×) (Gibco™ Thermo Fisher Scientific Inc., USA, Catalog No. 15240-062). The HCT116 cells were

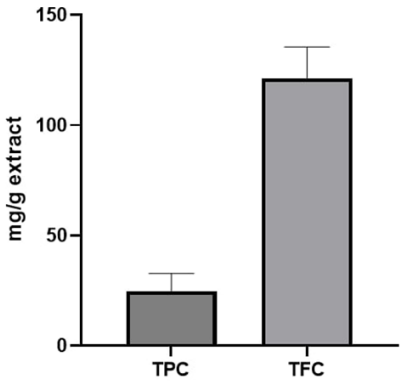

Figure 3. Total phenolic content (TPC) and total flavonoid content (TFC) of *Vaccinium uliginosum* crude leaf extract *V. uliginosum.*

cultured in McCoy's 5A medium (1×) (Gibco™ Thermo Fisher Scientific Inc., USA, Catalog No. 16600-082) supplemented with 10% heat-inactivated fetal bovine serum (Gibco™ Thermo Fisher Scientific Inc., USA, Catalog No. 10500-064) and 1% antibiotic-antimycotic solution (100×). Meanwhile, A549 cells were cultured in Ham's F-12K (Kaighn's) medium (Gibco™ Thermo Fisher Scientific Inc., USA, Catalog No. 21127-022) supplemented with 10% heat-inactivated fetal bovine serum, 1% sodium bicarbonate 7.5% solution (Gibco™ Thermo Fisher Scientific Inc., USA, Catalog No. 25080-094) and 1% antibiotic-antimycotic solution (100×).

2.9. Cell Viability Assay or MTT Assay

The MTT cytotoxicity assay was based from a previous protocol with modifications [19]. MRC-5, HCT-116, and A549 cells were seeded at 8,000 cells per well in sterile 96-well microtiter plates. Plates were incubated overnight in a humidified incubator at 37 °C and 5% CO_2 to allow cell attachment. Eight two-fold dilutions of the sample were used as treatments, starting from 100 down to 0.78125 µg/mL. Diluted doxorubicin and diluted DMSO were prepared at the same concentrations as the samples, serving as the positive and negative controls, respectively. Diluted DMSO was also used as a solvent for dissolving the samples and doxorubicin because it maintains the stability and bioactivity of the drug, ensuring effectiveness when exposed to cells. The final DMSO concentration in all wells, including control and treatment groups, was standardized to ensure consistency and to eliminate solvent-induced variability. This approach allows for precise control of the concentration of both the sample and the drug. After overnight attachment, cells were treated with the prepared concentrations of samples and controls and were again incubated for 72 h at 37 °C and 5% CO_2. After 72-h incubation, 40 µL of 0.5 mg/mL of 3-(4,5 - dimethylethylthiazol - 2 - yl) - 2,5 - diphenyltetrazolium bromide (MTT) solution (Amresco, USA, Catalog No. 0793) was added to all wells and incubated at 37 °C and 5% CO_2 for 3 h for both HCT-116 and A549 while 4 h for MRC-5 cells. After which, 150 µL DMSO (RCI Labscan, Thailand, Catalog No. AR1054) was used to dissolve the metabolized formazan crystals formed through the reduction of the MTT dye. Absorbances were read at 570 nm wavelength (OD570) using an Accuris SmartReader 96 Microplate Absorbance Reader (Accuris Instruments, USA). The cell viability of the cells was calculated using the following Equation 2.

$$\text{Cell viability rate (\%)} = \text{treated group/control group x 100\%} \quad (2)$$

IC_{50} values (µg/mL) were calculated using non-linear regression using GraphPad Prism v. 10.3.1 (Boston, MA 02110, USA) and were used to determine the cytotoxicity activity level of the extracts tested based on the criteria: IC_{50} value of > 10 µg/mL indicate highly toxic; 10–29.99 µg/mL indicate moderately toxic; 30–100 µg/mL indicate slightly toxic; and > 100 µg/mL indicate not toxic or proliferative or biphasic action according to the American National Cancer Institute (NCI) [20]. Photo documentation was performed for each concentration using an inverted phase-contrast microscope (Zeiss, Research Microscopy Solutions, Oberkochen, Germany) at 100× magnification.

2.10. Statistical Analysis

Statistical analysis was performed using GraphPad Prism v. 10.3.1 (Boston, MA 02110, USA). Statistical comparisons of cell viability assay between each concentration tested and a single control group were performed with one-way ANOVA followed by Dunnett's multiple comparisons post-hoc test. IC_{50} (µg/mL) values were calculated using non-linear regression (curve fit) formula for Absolute IC_{50} in GraphPad Prism v. 10.3.1 (Boston, MA 02110, USA). All data are presented as mean ± SEM. A p-value of < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical Screening of the Crude Leaf Extract of V. Uliginosum

The phytochemical screening of the crude leaf extract of *V. uliginosum* revealed the presence of several bioactive compounds, including alkaloids, flavonoids, steroids, saponins, and tannins (Table 1). The levels of these compounds are indicated by "+" signs, with single, double, and triple "+"

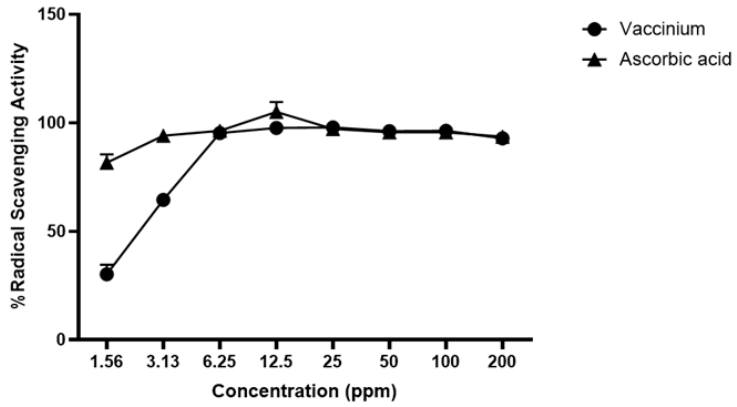

Figure 4. DPPH radical scavenging activity of *Vaccinium uliginosum* crude leaf extract. *V. uliginosum* with EC_{50} value of 2.85 ppm while ascorbic acid has EC_{50} value of 5.62 ppm. Data presented as mean±SEM.

denoting increasing levels of detection. Table 1 shows that flavonoids, saponins, steroids, and tannins were most abundant, represented by a triple "+" (+++), while alkaloids were present at lower levels (+), and anthraquinones and cyanogenic glycosides were absent. This suggests that the *V. uliginosum* leaf extract is rich in bioactive compounds with potential therapeutic effects. To quantify specific bioactive compounds, we conducted a TPC assay. Phenolic compounds include a wide range of bioactive molecules, such as flavonoids, phenolic acids, and tannins [21]. Our results showed that the plant leaf extract contains 24.58±8.11 GAE mg/g (Figure 3). Environmental conditions likely influence phenolic content, as shown in previous studies of *Vaccinium vitis-idaea L.* [22] and *Vaccinium corymbosum L.* [23], where phenolic content varied with climate. We further quantified the TFC of the crude leaf extract, which was found to be 121.38±14.04 QE mg/g (Figure 3), indicating its potential as a flavonoid source. The relatively higher flavonoid content compared to phenolic content can be attributed to the fact that phenols represent a larger group of compounds, with flavonoids being just a subgroup of phenols [24]. Flavonoid is well known for its antioxidant, anti-inflammatory, and anticancer properties [25], arresting the cell cycle [26], inducing apoptosis [27], and suppressing cancer cell proliferation and invasiveness [28]. Tannins, known for their anticancer, antioxidant, antimicrobial, and antibacterial properties [29], include compounds such as gallotannin, which has been shown to induce apoptosis in colon cancer cells [30]. Saponins, also abundant in the extract, are recognized for their anticancer [31], anti-angiogenesis [32], and anti-inflammatory effects [33]. They are also known to enhance chemotherapy by reversing multidrug resistance [34]. Steroids, another highly detected compound, are used clinically to treat leukemia and lymphomas [35][36], suggesting the extract's therapeutic potential for these diseases. Thus, the presence of these bioactive compounds—alkaloids, flavonoids, saponins, steroids, and tannins—in the *V. uliginosum* crude leaf extract supports its potential uses in treating various diseases, as traditionally believed by the IPs of Benguet Province.

3.2. Antioxidant Activity of the Methanolic Crude Leaf Extract of V. uliginosum

DPPH assay is a well-established method for evaluating the antioxidant capacity of plant extracts, which is based on the reduction of the DPPH radical, which causes a color change from purple to yellow [37]. Our results indicated strong antioxidant potential, with an EC_{50} value of 2.85 ppm, indicating high free radical scavenging efficiency [18]. At a concentration of 25 mg/mL, the extract exhibited 97.86±1.33% DPPH radical inhibition (Figure 4), which supports the observed antioxidant activity but should not be solely interpreted as the measure of antioxidant capacity. The high antioxidant activity is likely attributed to the extract's rich content of anthocyanins and polyphenols, as demonstrated in the TPC and TFC assays (Figure 3). These phytochemicals are known for their ability to neutralize free radicals and

protect biological molecules from oxidative stress [38][39].

The high antioxidant activity observed is consistent with previous studies on other *Vaccinium* species, such as *Vaccinium myrtillus* and *Vaccinium corymbosum*, which are known to contain abundant polyphenolic compounds, including delphinidin, cyanidin, and quercetin derivatives [40][41]. These compounds not only contribute to antioxidant defense but have also been implicated in chemopreventive mechanisms by modulating redox -sensitive signaling pathways and inducing apoptosis in cancer cells [42][43]. For instance, polyphenolic compounds such as flavonoids and anthocyanins, which are abundant in *Vaccinium* species, have been shown to exert anticancer effects through pathways involving oxidative stress induction, mitochondrial membrane depolarization, activation of caspase-dependent apoptosis, and cell cycle arrest at the G0/G1 or G2/M phase [44][45]. Flavonoids can also modulate signaling pathways such as PI3K/Akt, MAPK/ERK, and NF-κB, which are critical for cell survival, proliferation, and inflammation in cancer cells [46][47]. In particular, anthocyanins from bilberries and cranberries have been reported to induce ROS-mediated apoptosis in colorectal and oral cancer cells, possibly by downregulating anti-apoptotic proteins (e.g., Bcl-2) and upregulating pro-apoptotic markers (e.g., Bax, caspase-3) [48][49]. Given the similar phytochemical profile observed in *V. uliginosum*, these pathways may likewise be implicated in the extract's selective cytotoxic effects. The findings of the present study suggest that *V. uliginosum*, like its close relatives, possesses strong antioxidant potential and may have therapeutic relevance in mitigating oxidative stress-related diseases, including cancer. Further studies are warranted to isolate specific bioactive compounds and investigate their molecular targets.

3.3. Cytotoxicity Activity of the Leaf Extracts of V. uliginosum in Several Human Cell Lines

When tested against cancer cell lines, the methanolic extract of *V. uliginosum* exhibited slight toxicity towards HCT-116 cells, a colorectal cancer cell line, with an IC_{50} of 69.26±6.83 µg/mL, but it was non-toxic and proliferative against A549 cells, a lung adenocarcinoma cell line, with an IC_{50} greater than 100 µg/mL (Figures 5(A) and 5(B)). Upon partitioning into *n*-hexane, the extract showed moderate toxicity against HCT-116 cells, with an IC_{50} of 27.99±5.51 µg/mL, and slight toxicity against A549 cells, with an IC_{50} of 65.79±1.52 µg/mL (Figures 5(C) and 5(D)). Conversely, the ethyl acetate extract was non-toxic but induced proliferation in A549 cells, with an IC_{50} of 130±13.97 µg/mL. This plant extract displayed a biphasic activity against HCT-116 cells, where it was toxic at the highest concentration but proliferative at the lowest concentration, although this effect was not statistically significant (Figures 5 (E) and 5(F)). These findings suggest that the n-hexane fraction of *V. uliginosum* has the most promising cytotoxic activity against HCT-116 and A549 cancer cell lines, which could be attributed to the presence of bioactive compounds with selective anticancer properties.

Morphological observations revealed distinct responses of the three cell lines (MRC-5, HCT116, and A549) to the methanolic, *n*-hexane, and ethyl acetate extracts. In MRC-5 normal lung fibroblast cells, both methanolic and ethyl acetate extracts showed no apparent cytotoxic activity at any concentration; cells maintained normal morphology and viability across all doses. In contrast, the *n*-hexane extract exhibited marked cytotoxicity at higher concentrations, with extensive cell rounding, detachment, and features resembling apoptosis, closely mimicking the positive control (doxorubicin). At lower concentrations of the *n*-hexane extract, MRC-5 cells remained viable and even appeared visually proliferative. In HCT116 colon carcinoma cells, the methanolic extract induced some degree of cell shrinkage and rounding at higher concentrations, although a portion of cells remained intact. The n-hexane extract, however, caused significant morphological signs of apoptosis at higher doses, including cell rounding and detachment, similar to the positive control, while at lower doses, despite some cell death, a notable population of proliferating cells was observed. The ethyl acetate extract had no visible cytotoxic effects on HCT116 cells, with cells retaining a normal appearance across concentrations. Similarly, in A549 lung carcinoma cells, both the methanolic and ethyl acetate extracts showed no morphological signs of cytotoxicity, with cells maintaining

viability at all tested concentrations. The *n*-hexane extract led to observable shrinkage and rounding of cells, indicative of stress or early apoptosis, although a number of viable cells persisted. These findings suggest that among the tested extracts, the n-hexane fraction exerted the most potent cytotoxic effects, particularly at higher concentrations, while methanolic and ethyl acetate extracts were largely non-toxic under the tested conditions.

These results are consistent with previous research on other species within the *Vaccinium* genus. For example, one study demonstrated the anti-proliferative effects of Andean berry juice against colorectal cancer, indicating potential avenues for cancer treatment [50]. Another study highlighted the medicinal properties of Bilberry fruit (*Vaccinium myrtillus* Linn.), potentially serving as an antidiabetic and antioxidant source due to its chemical composition [6]. Angelova's team [50] showed the antitumor potential of Lingonberry (*Vaccinium vitis-idaea* L.) on cervical and breast cancer cells, emphasizing a dose-dependent inhibitory effect and induction of apoptosis. Further research by Ankola et al. [51] investigated the cytotoxicity of *Vaccinium macrocarpon* (cranberry) extract on oral cancer cells, indicating its potential against cancer cell proliferation. Additionally, black raspberry has been shown to induce apoptosis in oral squamous cell carcinoma cells, with effects comparable to the anticancer drug doxorubicin [52]. These comparative findings reinforce the anticancer potential of *V. uliginosum* and its relevance within the broader *Vaccinium* genus, which is rich in

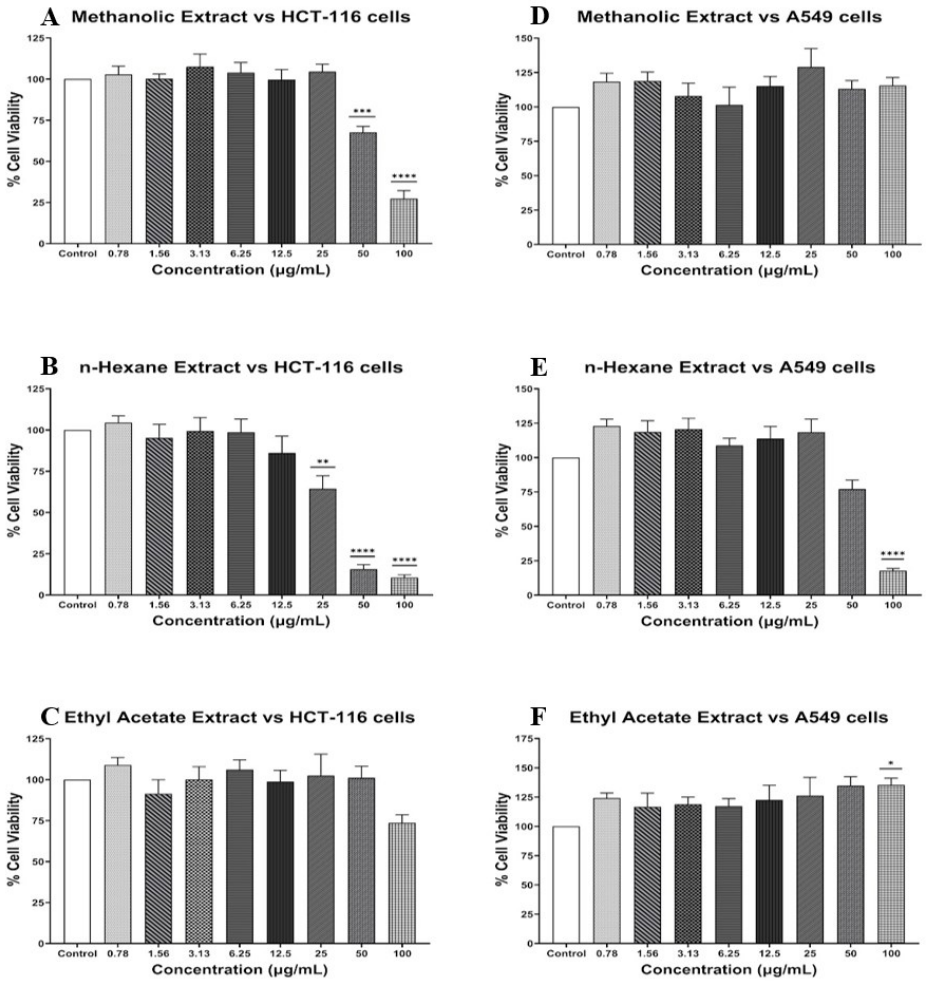

Figure 5. Cytotoxicity activities of *Vaccinium uliginosum* crude extract against HCT-116 and A549 cancer cell lines, measured using the MTT assay after 72 hours of treatment. Percent cell viability of HCT-116 cancer cells treated with (a) methanolic, (b) *n*-hexane, and (c) ethyl acetate extracts; and A549 cancer cells treated with (d) methanolic, (e) *n*-hexane, and (f) ethyl acetate extracts. Data presented as mean±SEM from three independent trials. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ vs. control.

phytochemicals such as anthocyanins, flavonoids, and polyphenols known for their cytotoxic and chemopreventive properties.

The cytotoxic activity of *V. uliginosum* was evaluated using the MTT assay against various human cell lines, including MRC-5, HCT-116, and A549. MTT assay is a method employed to assess the viability of cells and their growth modulation *in vitro* [19]. This assay also serves as a cost-effective and reliable tool, offering a straightforward means to test the cytotoxic activity of *V. uliginosum*. MRC -5 cell line is a diploid cell culture line with a fibroblast-like morphology, commonly used in the production of several vaccines including for hepatitis A, varicella and polio [53]. The HCT-116 cell line is a well-characterized human colorectal carcinoma cell line frequently used in cancer research to study tumor progression and therapeutic responses [54]. While the A549 cell line, derived from human lung adenocarcinoma, is widely used in studies related to lung cancer biology and the testing of potential anticancer agents [55]. The IC_{50} of each extract against A549 and HCT-116 cells was calculated following the MTT assay. The IC_{50} value, is used indicates the concentration required to inhibit the growth or viability of cells by 50% [56]. Therefore, the IC_{50} values obtained in this study provide insights into the cytotoxicity of *V. uliginosum*, as they reveal the potency of its fractions in inhibiting the growth of HCT-116 and A549 cells.

The cytotoxic activity of these extracts was tested against the MRC-5 cell line, a normal human fibroblast cell line. Our results demonstrated that only the *n*-hexane extract of *V. uliginosum* exhibited slight cytotoxic activity against MRC-5 cells, with an IC_{50} of 56.37±0.96 µg/mL (Figure 6 (B)), compared to the positive control (Figure 6 (D)). In contrast, the methanolic and ethyl acetate extracts displayed dose-dependent proliferation activities, with IC_{50} values greater than 100 µg/mL (Figures 6(A) and 6(C)), indicating low cytotoxicity and potential proliferative effects.

The observed differences in cytotoxic activity between the *n*-hexane, ethyl acetate, and methanol extracts of *V. uliginosum* can be attributed to the solvent-specific extraction of distinct phytochemical classes. Non-polar solvents such as *n*-hexane tend to extract lipophilic compounds, including terpenoids and fatty acids, which may exert non-selective cytotoxic effects by disrupting cell membranes or mitochondrial integrity in both cancer and normal cells. In contrast, methanol, a highly polar solvent, extracts hydrophilic compounds such as polyphenols, flavonoids, and anthocyanins, some of which are known to induce apoptosis selectively in cancer cells through pathways involving oxidative stress, mitochondrial depolarization, or caspase activation. Ethyl acetate, with intermediate polarity, may extract moderately polar compounds such as flavonoid aglycones or coumarins, which also exhibit diverse bioactivities. This variation in cytotoxic profiles highlights the pharmacological complexity of the plant and the critical role of solvent polarity in modulating biological activity.

Interestingly, while the *n*-hexane extract exhibited the highest cytotoxic activity against HCT -116 colorectal cancer cells, it also showed toxicity toward normal MRC-5 fibroblasts. In contrast, the methanolic extract demonstrated selective cytotoxicity, significantly inhibiting the proliferation of HCT-116 cells while exhibiting minimal or no cytotoxic effects on MRC-5 cells, even at higher concentrations. This cancer-specific activity suggests that certain bioactive compounds present in the methanolic extract may selectively target tumor cells. Such selectivity is a desirable feature in anticancer drug development, as it reduces the risk of adverse effects on healthy tissues. Therefore, the methanolic extract of *V. uliginosum* may represent a promising candidate for further studies on cancer-specific therapeutics.

These results suggest that the observed antioxidant activity is linked to the high content of polyphenols and flavonoids in the extract, which are known for their free radical-scavenging capabilities and protective effects against oxidative damage [57] [58]. The slight cytotoxicity observed in the MRC-5 normal cells, compared to the more pronounced effects on cancer cells, suggests a degree of selectivity, as the extract was more toxic to cancer cells than to normal cells. The biphasic response with the ethyl acetate extract might indicate the presence of compounds with dose-dependent effects, which can act as either antiproliferative agents at higher doses or growth stimulators at lower doses. This phenomenon is often linked to the

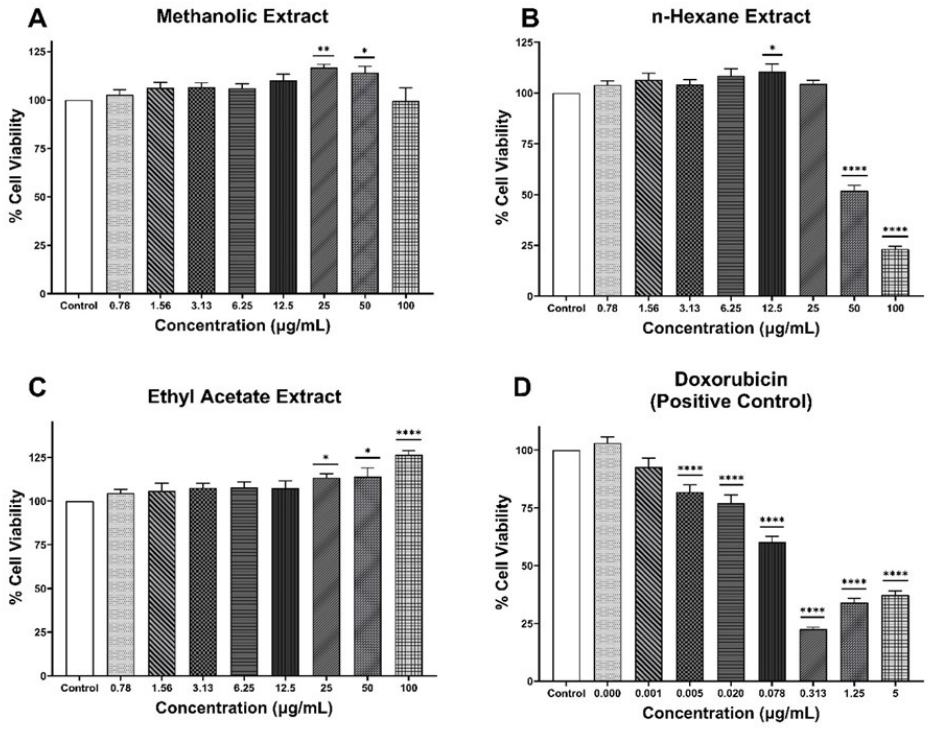

Figure 6. Cytotoxicity activities of *Vaccinium uliginosum* crude extract against normal cell line, MRC-5, measured using MTT assay for 72h. % cell viability after MRC-5 cell lines are treated with (a) methanolic; (b) *n*-hexane; (c) ethyl acetate extracts, and (d) positive control doxorubicin. Data presented as mean±SEM from three independent trials. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 vs. control.

complex interactions of phytochemicals within plant extracts [39][59]. The cytotoxicity profile observed was biphasic in nature: while high concentrations of the compound induced pronounced cytotoxic effects, lower concentrations were associated with a modest but reproducible increase in cell proliferation. This biphasic response is indicative of a hormetic effect, wherein sub-toxic doses may activate cellular survival or proliferative pathways, highlighting the importance of dose in modulating biological outcomes.

Biphasic properties, as exhibited by the *V. uliginosum* plant extracts, refer to the phenomenon where a substance produces different effects at varying concentrations—typically stimulatory or proliferative at lower concentrations and inhibitory or cytotoxic at higher concentrations. This dual activity is often attributed to the complex nature of phytochemicals present in plant extracts, where multiple compounds may interact synergistically or antagonistically, leading to dose-dependent outcomes. For instance, certain flavonoids and polyphenols, which are abundant in *V. uliginosum*, have been shown to exhibit antioxidant effects at low doses but can induce pro-oxidant effects, leading to cytotoxicity, at higher concentrations [59]. This biphasic behavior is also observed in other bioactive compounds, such as saponins and alkaloids, which may promote cell proliferation at low levels due to their mild stress-inducing properties that activate survival pathways, while at higher concentrations, they overwhelm cellular defense mechanisms, leading to apoptosis [39]. The biphasic nature of *V. uliginosum* extracts indicates the importance of dosage in therapeutic applications, as the beneficial or harmful effects of the plant extract can vary significantly depending on the concentration used. This characteristic highlights the need for careful dose optimization in the potential development of *V. uliginosum*-derived therapies.

4. CONCLUSIONS

The present study demonstrates that *V. uliginosum* crude leaf extracts exhibit significant anticancer and antioxidant activity, possibly due to compounds like anthocyanins and polyphenols. *V.*

uliginosum crude leaf extracts, particularly at higher concentrations when partitioned using an n-hexane solvent, showed a promising cytotoxic activity against cancer cells. Among the cell lines tested, *V. uliginosum* showed moderate toxicity against HCT-116 cancer cells, but also showed slight toxicity against A549 and MRC-5 cells. The extract's toxicity to normal human lung fibroblasts highlights the complexity and raises concerns about its suitability as a stand-alone chemotherapeutic alternative. Therefore, further research is necessary to isolate and characterize the specific bioactive compounds responsible for its effects and to investigate their mechanism of action, therapeutic efficacy, and safety in more comprehensive biological models. This will help determine the true potential of *V. uliginosum* in cancer therapy and its possible integration into current treatment regimens. This study contributes to the advancement of the United Nations Sustainable Development Goals (SDGs), particularly SDG 3: Good health and well-being, by exploring the anticancer potential of *V. uliginosum* leaf extracts. Through investigating natural bioactive compounds for their cytotoxic and antioxidant properties, this research supports efforts to promote health, develop safer and more effective cancer treatments, and reduce the global burden of disease. Additionally, the work aligns with SDG 12: Responsible consumption and production, by highlighting the value of plant-based resources in biomedical applications and encouraging the sustainable use of biodiversity.

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Conflicts of Interest

The authors declare no conflict of interest.

ETHICS APPROVAL

All necessary permits such as certificate of precondition (CPIKSP-CAR-2022-017) issued by the National Commission on Indigenous People (NCIP) ethics approval from the University of Mindanao Ethics Review Committee (UMERC-2022-289)), and a wildlife Gratuitous Permit (GP) from the Department of Environment and Natural Resources, Cordillera Administrative Region (DENR-CAR-08-2022) were obtained. The authors asked permission from the local authorities, community elders, and the indigenous people.

SUPPORTING INFORMATION

Supplementary data associated with this article can be found in the online version at doi: 10.47352/jmans.2774-3047.305

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DECLARATION OF GENERATIVE AI

Not applicable.

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