Original Article

# Antioxidant and Antitumor Properties of Wild Blueberry (Sideroxylon mascatense): Effects of Drying Methods

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## Abstract

**Background:** *Sideroxylon mascatense* is a wild blueberry plant that traditionally known as "boot" in Oman. Studies on health beneficial effects of this fruit are very limited. **Aims and Objectives:** This study aimed to evaluate the antioxidant and antitumor properties of *Sideroxylon mascatense* under different drying methods. **Materials and Methods:** Freeze (−20°C) and air drying (60 and 90°C) methods were used. The antioxidant potential properties were evaluated using DPPH and ABTS assay. Moreover, the antitumor activities of blueberry extracts were determined using Alamar blue assay. **Results:** The results of this study revealed that a blueberry extract at 20 mg powder/mL provided the maximum amount of vitamin C, reaching a value of 19.45 mg/100 g dry-basis in the flesh dried at 60°C and 14.24 mg/100 g dry-basis in seed dried at 90°C. The freeze-dried blueberry exhibited the highest percentage of free radical scavenging activity (DPPH) from both fruit flesh (50.8%) and seeds (39.4%) at a concentration of 20 mg/mL indicating a higher antioxidant activity. There was no significant difference in the ability of blueberry extracts to scavenge radical cations ($ABTS^+$) at various concentrations of extracts. However, there was a 40% reduction of $ABTS^+$ radical formation in blueberry flesh extracts obtained from freeze-dried berry at a concentration of 20 mg/mL. On the other hand, blueberry seeds showed variation in the scavenging activity of the $ABTS^+$ radical of 38% after different processing methods. Breast MCF7 and ovarian Ov2008 tumor cell lines were the most sensitive to the dried blueberry. The 50% growth inhibition (IC50) of the freeze-dried blueberry extract at both cell lines was 64 and 69 μg/mL, respectively. **Conclusion:** Our findings indicated a potent antioxidant activity of *Sideroxylon mascatense*, but further research is required to isolate the active compounds in the fruit and test their effects in an experimental animal model.

**Keywords:** Antioxidants, cancer, drying methods, sideroxylon mascatense

## Introduction

The Arabian Gulf region is considered the homeland of many traditional and herbal remedies. There are approximately 3500 known species in the flora of the Arabian Peninsula, with approximately 2000 species in Saudi Arabia and about 2800 species in Yemen, followed by Oman.[1] The total number of species in Oman includes 1204 wild plants, many of which are utilized as traditional medicine. Less than 10% of the wild species in the Arabian Gulf region have been screened for their therapeutic use, and there are very limited studies on the wild edible plants in Oman.[2-6] *Sideroxylon mascatense* (locally known as "boot") is a wild berry plant that grows between 1400 m and 2400 m in altitude, and is difficult to harvest.[7,8] The fruit turns blue or purple when ripe and is edible with a sweet, slightly acidic taste, and a single small pit fills the inside of the fruit.[8]

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Different varieties of wild berries are reported worldwide and with a distinct color, size, flavor, chemical composition, and bioactive content of the fruit.[9]

Nowadays, consumer's interest towards lesser-known wild edible fruits has increased rapidly due to their various health beneficial effects along with exotic organoleptic characteristics such as blueberries.[6] Furthermore, it contain a wide variety of

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phenolic compounds such as anthocyanins, proanthocyanidins, phenolics, and flavonols.[10-15] Therefore, wild blueberries consumption increased globally since 2014 at higher rate compared to the high-bush blueberries.[16] Moreover, it is used in different processed food such as jams, juices, jellies, yoghurt. Wild blubbery showed an improvement in cognitive performance in children[17] and therefore, it has been incorporated in different types of food (i.e. oatmeal bar, beverage, ice pop, gummy, and cookie) to encourage children to consume wild blueberries and become part of their dietary patterns.[14]

There is a strong evidence supporting the high phenolic content as well as the antioxidant capacity of wild berries as compared with those domesticated and genetically modified crops.[18] Wild blueberries species such as *Vaccinium angustifolium* Ait. and *Vaccinium oldhamii* Miq. showed a significant antioxidant activity.[11-13]

The phenolic compounds exhibit a wide range of therapeutic effects with potential benefits due to the presence of natural antioxidants and micronutrients which may contribute to limit the development of several diseases including anti-aging, fever-suppressing, anti-inflammatory, anti-diabetic, painkilling, detoxifying, obesity, reduce total cholesterol LDL and HDL and anticancer.[15,18-21] For instance, wild blueberry (*Vaccinium angustifolium*) inhibited the initiation stage of chemically-induced carcinogenesis[12] play a protective role against neurodegenerative disorders.[13] Also, it reduced the levels of oxidized DNA bases and increased the resistance to oxidatively induced DNA damage.[22] Moreover, it was found that supplementing rat's diet with 2% of blueberry extracts for 8 weeks protected them against neurodegeneration and cognitive defects caused by the oxidative process.[23]

There are also many studies that report the effectiveness of berry fruits as anticancer both *in vivo* and *in vitro*, and bioactive compounds of blueberry have significant anticancer effects through various complementary mechanisms of action, including the induction of metabolizing enzymes and the modulation of gene expression; and their effects on cell proliferation, apoptosis, and subcellular signaling pathway.[24,25] *In vitro* studies indicated anti-proliferative activity of different kinds of blueberries on SW48 and HT-29 cancer cell lines and liver HepG2 cancer cells.[26,27]. Furthermore, Natsuhaze wild blueberry (*Vaccinium oldhamii* Miq.) was very effective in inhibiting the growth of HL-60 human leukemia cells in *vitro* compared to other wild and cultivar species.[11] Other types of cancers has been studied (i.e. using cell line) using wild blubbery such as prostate cancer[10] and oral cancer.[28] The effect of blueberries on ovarian cancer and breast cancer was investigated as ovarian cancer considered as the sixth and eighth leading cause of cancer mortality among women in developed and developing countries, respectively"[29] whereas breast cancer is the most frequent cancer among women.[30] Studies showed that blueberry inhibited the proliferation of ovarian cancer[29] and growth of breast cancer.[31-32]

With approximately 40% of the world population will be diagnosed with cancer during their lifetime, studies indicated that blueberries can be a potential therapy due to their anti-carcinogenic properties.[33] Moreover, the therapeutic level is dose depends and more studies are required. Therefore, the main objective of this study was to assess antioxidant properties and antitumor activity on breast and ovarian tumor cell lines of the fruits *Sideroxylon mascatense* under different drying methods.

## Materials and Methods

### Chemicals

Folin Ciocalteu reagent (Merck, Germany), sodium carbonates (7.5%), sodium hydroxide (1M), sodium nitrite solution (5%), and aluminum chloride solution (10%) were of analytical grade (Sigma, St. Louis, MO, USA). Ascorbic Acid Assay Kit (k671), ABTS Antioxidant Assay Kit (Zenbio, Cat#AOX-1) were purchased from Biovision Company, USA.

### Preparation of blueberry fruit extract

Omani wild blueberry *Sideroxylon mascatense* was purchased from Al-Jabal Al-Akhdar (Sultanate of Oman) during peak season in August 2016. Samples were packed in plastic bags and directly placed in a cool box. These fruits were transferred to the laboratory and rapidly frozen and stored at −60°C until used for further analyses. The edible parts of the fruits were separated from seeds. Both flesh and seeds were dried in an air oven at two different temperatures 60°C for 22 h and then 90°C for 8 h or freeze-dried for 5 days using a benchtop laboratory freeze drier (Labconco, USA). The shelf temperature was −40°C, and chamber temperature was at 20°C with an operating pressure of 200 Pa. The condenser temperature of the vacuum exit was at −80°C. The dried samples were then ground to a fine powder using a household grinder (Moulinex AR1043-UK0, Moulinex, Lyon, France). Three extracts were prepared as follows: 0.5, 1.0, and 2.0 g of the dried powder from both flesh and seed were extracted with 100 ml of distilled water at 20°C for 2 h using a magnetic stirrer. The extracts were then filtered and centrifuged at 6000 rpm for 20 min at 4°C. The supernatant was collected, and this crude extract kept in dark plastic bottles at −60°C until used for further analysis. Extracts were analyzed in three analytical replicates. These extracts were considered labelled as 5 mg/ml, 10 mg/ml, 20 mg/ml, respectively.

### Total antioxidant potential properties

The below assays were conducted within the context of highlighting the antioxidant potential properties of the blueberry fruit extract.

#### *Vitamin C assessment*

Ascorbic acid content was measured using BioVision's Ascorbic Acid Assay Kit (K671). Briefly, a proprietary catalyst oxidizes ascorbic acid to produce a compound that

interacts with the ascorbic acid probe, generating a color that was quantified by colorimetry (spectrophotometry at =570 nm, Thermo Scientific Fluoroskan Ascent, USA) following the recommendations of the manufacturer.

### Free radical scavenging capacity by DPPH assay

The capacity of the extracts to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was measured by spectrophotometry. Briefly, 50 µL extract was mixed with 50 µl of a DPPH methanolic solution (0.04 mg/mL). Absorbance was measured at 517 nm after 30 min of incubation at room temperature.[34] The 2,6-di-tert-butyl-4-hydroxytoluene (BHT) used as a positive control with a 100% reduction for DPPH free radical formation.

### ABTS antioxidant assay

BioVision's ABTS Antioxidant Assay Kit (Zenbio, Cat#AOX-1, USA) was used. The assay is based on the incubation of an extract (s at different concentrations (5–300 µg/mL) with 2, 2′-azino-di-[3-ethylbenzthiazoline sulphonate] (6) diammonium salt (ABTS). Peroxidase (methmyoglobin) and hydrogen peroxide produce the radical cation ABTS+ which was relatively stable blue-green color, and absorbance was measured at 405 nm.[34]

### Antitumor activity of blueberry extracts

Breast cancer cell lines, MCF-7 and MDA-MB-231, were purchased from the American Type Culture Collection (ATCC, USA), while human adult fibroblast cells were purchased from Science Cell Research Laboratories (San Diego, Ca, USA). Ovarian tumor cell lines, OV2008 and OVSAHO, were provided by Dr. Aikou Okamoto from The Jikei University School of Medicine in Japan. Dulbecco's Modified Eagle Medium (DMEM) (Gibco®, Life Technologies, CA) supplemented with 10% FBS (Fetal Bovine Serum) (Invitrogen TM, Life Technologies, CA) and 1% penicillin–streptomycin (Invitrogen TM, Life Technologies, CA) was used to culture the cell lines (Fibroblasts, MCF-7, MDA-MB-231, OV2008 and OVSAHO) as described earlier (31). Briefly, cells were taken out of the liquid nitrogen and thawed in a 37°C water bath for one minute, then centrifuged for 30 seconds, and the supernatant containing DMSO was withdrawn. Cell pellets were immediately resuspended in the corresponding media and seeded in appropriate flaks. Cells were propagated in a 37°C humidified incubator with a 5% $CO_2$ atmosphere for 24 hours before media refreshment and left for growth. Cells reaching ~80 confluency were subjected to trypsin (0.25%) treatment until complete detachment, then pelleted and recovered in fresh media before seeding in 96 well plates (~$10^5$ cells/well).

### Preparation of blueberry extracts

The viability of the cells after treatment was tested in 96 well plate using different concentrations of blueberry extracts (1, 3, 5, 10, 15, 30, 45, 50, 75, 100, and 120%). So that, a stock solution of 5 mg of the obtained different blueberry extracts was prepared by dissolved in 5 mL DMSO (100%). In the 96 well plates, column 12 was used for control. While different concentrations of blueberries were distributed in different columns in which different rows were used for 60 °C and 90 °C air-dried samples and for freeze-dried berry at −40°C.

**Table 1: The half maximal inhibitory concentration (IC50) of the blueberry extracts against tumor cells growth *in vitro***

| | The 50% growth inhibition (IC50) of the blueberry extract | | |
|---|---|---|---|
| Tumor cell lines | Freeze dry−20°C | Air dry60°C | Air dry90°C |
| MCF-7 | 64.0 | 64.0 | 60.0 |
| MDA-MB-321 | 83.5 | 88.0 | 89.0 |
| OVSAHO | 79.2 | 70.0 | 81.0 |
| OV2008 | 69.0 | 64.0 | 60.0 |

### Treating cells with blueberry extracts

Cells were treated with increasing doses of the blueberry extracts diluted in the growth media [Table1]. Vehicle controls included dilutions of DMSO, and media were used. Vehicle controls were included to indicate the possible death of cells by the product and not by the solvent. They were loaded in well 11. Control included media alone was used in well 12. The plates were then incubated for 24 h. Thereafter, the cells were treated with 5% Alamar Blue.

### Alamar blue assay

Alamar Blue (AB) assay was performed to determine cell viability test because it is water-soluble, permeable through cell membranes, non-toxic to cells, and extremely stable in culture medium. Resazurin is the active component of the AB assay, which gets reduced to Resourfin by mitochondrial enzymatic activity in the cell cytosol. This is the principle behind the change in color of the cell medium from blue to a fluorescent pink that indicate that the cells are alive. On the other hand, dead cells will maintain the original blue color. Therefore, visual analysis of the color shows whether the cells are dead or alive. 5% AB was prepared by diluting 500 µL of AB dye with 9.5 mL growth media. A volume of 100 µl AB was added to each well and the plates were incubated for a period of 4 h. Quantification of the viable cells was done using a micro-plate reader.

### Statistical analysis

The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Turkey's test using Graph Pad Prism (version 5.03; GraphPad Software Inc. San Diego, CA, USA). Chemical analysis analyzed in three replicates, and results was expressed as mean

values ± standard deviation (SD). Differences among chemical composition with different methods were analyzed using the least significant (LSD) test at the significance level of ($P<0.05$).

## Results and Discussion

### Antioxidant potential properties of blueberries

Blueberries as a natural product helps to fight the effects of oxidative stress leading to chronic diseases such as heart disease, diabetes, and certain cancers.[9] The concentration of vitamin C is known to be highly affected by a variety of environmental factors, including oxygen, pH, light, temperature, and moisture content.[35]

In our study the effect of the blueberry extracts was notable in a dose-dependent manner, and it was observed that there are significant differences in vitamin C content between flesh and seed at different drying methods, as shown in Figures 1 and 2. The blueberry extract (20 mg powder/mL) provided the maximum amount of vitamin C. The extract of blueberry flesh that had been dried at 60°C had a concentration of vitamin C of 19.45 mg/100 g dry-basis. The seeds also had a significant amount of vitamin C that contributed to the antioxidant activity. The extract with concentration (20 mg/mL) of blueberry seeds dried at 90 °C showed the maximum concentration of vitamin C (14.24 mg/100 g dry-basis).

Previous studies have shown that that small fruit of wild raspberry (*Rubus hirsutus* Thunb.) contains 16.33 mg/100 g fresh weight of ascorbic acid.[19] Ascorbate concentrations varied between different species of Vaccinium: *Vaccinium corymbosum* L (Highbush blueberry), *Vaccinium ashei Reade* (Rabbiteye) and *Vaccinium angustifolium* (Lowbush blueberry) exhibited 10.2 mg/100 g, 8.4 mg/100 g and 16.4 mg/100 g fresh weight of vitamin C respectively.[8] The variability between cultivars and species positively affect vitamin C content.[8] If the skin was damaged, ascorbic acid was released and could be oxidized, and the concentration in the fruit significantly reduced. A study conducted in freeze-dry raspberry found that vitamin C was present in the amount of 88.81 mg/100 g fruit. [36] In contrast, our study results revealed that, the freeze-dried blueberry sample revealed a low amount of ascorbic acid (7.59–9.73 mg/100 g fresh weight) in comparison to the dried sample. This might be attributed to that vitamin C was susceptible to significant loss during postharvest handling, processing, and storage condition.[36]

### Free radical scavenging capacity by DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is one of the most commonly used methods to evaluate antioxidant activity. DPPH free radical is relatively stable and can be reduced only by reactive compounds, such as phenolic compounds.[37] The capacity of scavenging free radicals of polyphenol components obtained from both flesh and seed of blueberry varied with drying techniques [Figures 2–4]. Treatment with blueberry extracts revealed that the DPPH free radical scavenging activity was increased significantly with extract concentrations, $P<0.05$. The extract of freeze-dried blueberry from both flesh (50.76%) and seed (39.37%) showed the highest % of free radical scavenging activity at concentration 20 mg/mL indicating higher antioxidant activity.

The berries flesh and seeds maintained a 12.2–21.32 % and 12.9–16.38 % scavenging ability, respectively, at a concentration of 5mg/L. Li *et al*.[15] reported that the DPPH free radical scavenging activity of wild blueberry (*Vaccinium* fruits) was 34.13%, which is comparable to our result in which blueberry seed showed 39.37% of DPPH reduction. In addition, a study conducted on Chinese wild raspberry (*Rubus hirsutus* Thunb.) showed their ability to scavenge the DPPH radical by 77.11%.[19] These significant differences in scavenging activity among types of blueberry fruits are greatly influenced by growing environmental factors, stage of ripeness of the fruit, harvesting, and storage conditions.[36] On the other hand, the thermal treatment may also affect the stability of the phenolic compounds and thus the antioxidant activity. It is

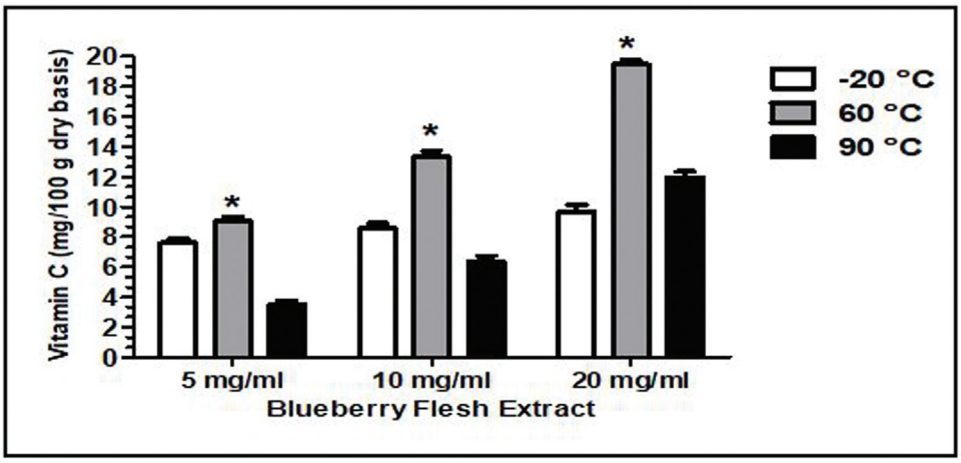


**Figure 1:** Vitamin C concentrations of blueberry flesh extracts at different drying temperature. *Significantly higher as compared to other groups, $P < 0.05$.

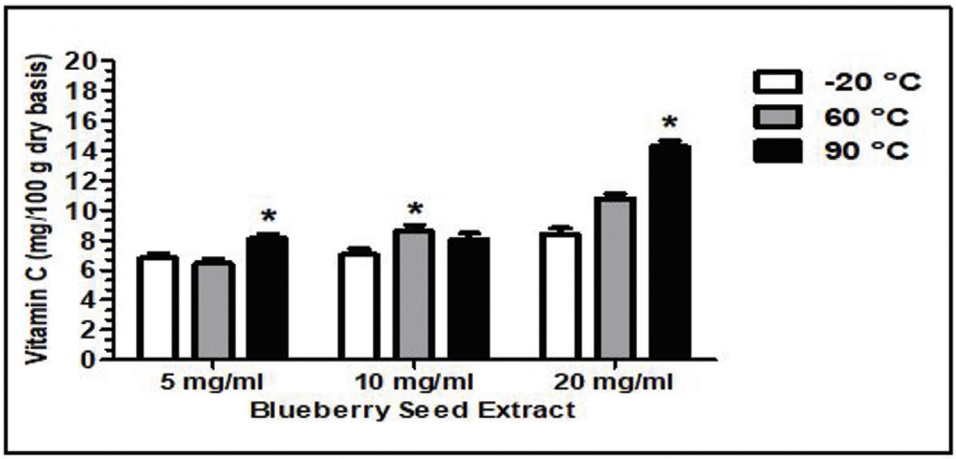


**Figure 2:** Vitamin C concentrations of blueberry seed extracts at different drying temperature. *Significantly higher as compared to other groups, $P < 0.05$.

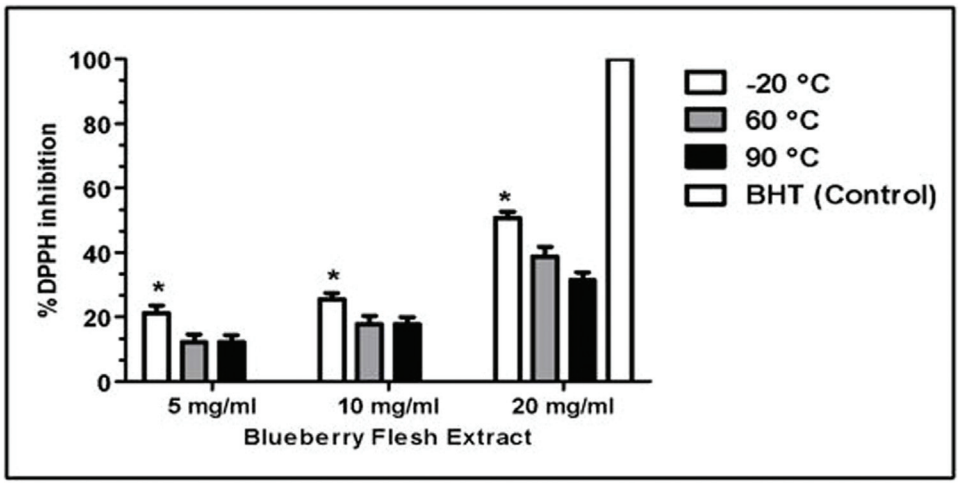


**Figure 3:** DPPH free radical scavenging activities of blueberry flesh extracts Butylated hydroxytoluene (BHT), a positive antioxidant control, with 100% inhibition for DPPH, 2,2-diphenyl-1-1-picrylhydrazyl, free radical formation. *Significantly higher as compared to other groups, $P < 0.05$.

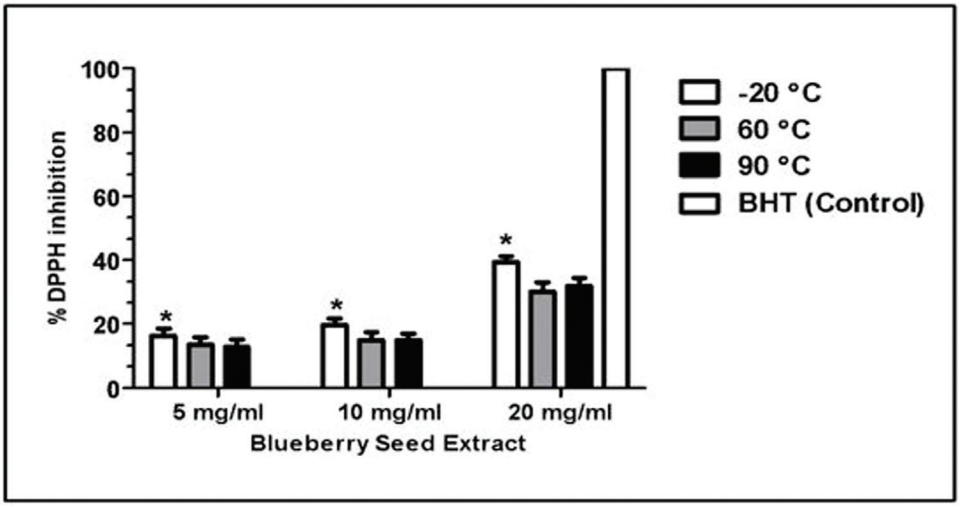


**Figure 4:** DPPH free radical scavenging activities of blueberry seed extracts Butylated hydroxytoluene (BHT), a positive antioxidant control, with 100% inhibition for DPPH, 2,2-diphenyl-1-1-picrylhydrazyl, free radical formation. *Significantly higher as compared to other groups, $P < 0.05$.

clear that vitamin C of the investigated wild blueberry samples (*Sideroxylon mascatense)* increased with the increase in drying temperatures. However, blueberry flesh dried at 60°C and seeds dried at 90°C had higher Vitamin C content than the other groups, $P<0.05$.

## ABTS antioxidant assay

Blueberry flesh, as well as seed scavenging activity of the ABTS radical after applying different dehydration processing methods, are presented in Figures 5 and 6. Trolox, vitamin E analog, acted as a positive control with 85% inhibition of ABTS radicals. The results reveal that there was no significant difference at various concentrations of blueberry extracts in both flesh and seed on their ability to scavenge the radical cation (ABTS+). However, there was a 40% reduction of ABTS+ radical formation in blueberry flesh extracts obtained by freeze-dried berry at a concentration level of 20 mg/mL. On the other hand, seeds have shown scavenging activity of the ABTS radical by 38% after different processing methods. This result may be explained the correlation between antioxidant activity of blueberry fruits and the content of total polyphenols and anthocyanins. Substantial loss of polyphenols content in dried blueberries corresponded to reduced affinity to scavenge both DPPH radical and ABTS radicals.[38]

In a study conducted by Kwok *et al*.[39], the effects of the different drying methods on the retention of Saskatoon berry phenolics and associated antioxidant activity were compared. The results clearly indicate that freeze-dried berry produced higher ABTS scavenging activities compared to other dehydration methods, with 65% retention of the original unprocessed fresh extract. The airdried berry had the lowest retained ability to scavenge ABTS radical (24%). A research study reported that dried Rabbiteye blueberry yielded 127.29 μM TE/ mL dry weight (DW) of ABTS compared to the fresh fruits that had a much higher ABTS scavenging activities, 236.74 μM TE/ mL DW.[40]

## Antitumor activity

The effect of the blueberry extracts on cancer cell lines was dose-dependent and varied with the cell type and the concentration of the berry extract. Increasing doses of berry extracts (1–120%) were used to determine the sub-optimal dose that remained effective in inhibiting the

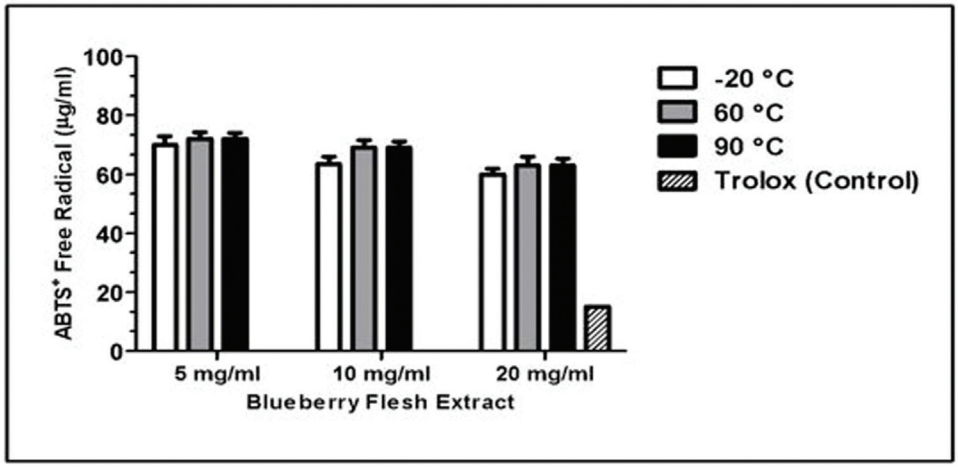


**Figure 5:** Antioxidant capacity of blueberry flesh extracts against ABTS (2,2'-azinobis 3-ethylbenzothizoline-6-sulfonic acid) radical formation.

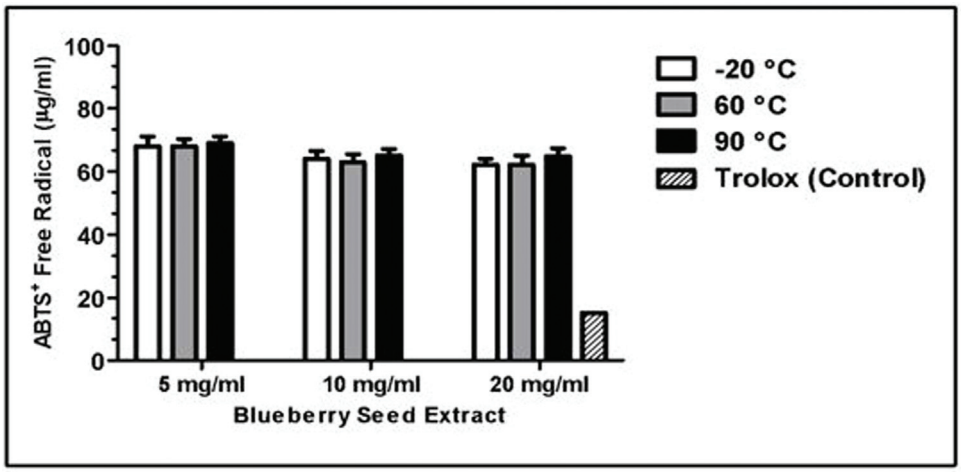


**Figure 6:** Antioxidant capacity of blueberry seed extracts against ABTS (2,2'-azinobis 3-ethylbenzothizoline-6-sulfonic acid) radical formation.

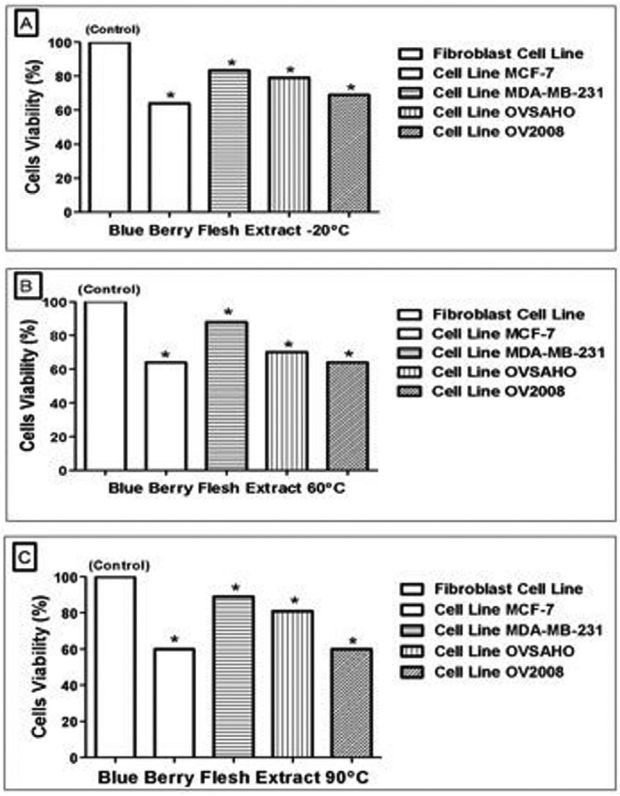


**Figure 7:** A,B,AC Anti-tumor effects of different blueberry flesh extracts using five different aggressive breast and ovarian human cell lines. *All extracts have significantly reduce the cells viability of the tumor cells as compared to control fibroblast cells.

proliferation of breast cancer cell lines. The concentration 0.75 μg/mL of blueberry was the most effective. Therefore, $IC_{50}$ was calculated at that concentration, and the blueberry extract at different drying temperatures selectively kill all four aggressive tumor cells (Fig. 7 A, B, C). The freeze-dried (−20°C) *Sideroxylon mascatense* had the strongest effect. It revealed a marked decrease in the viability of the breast and ovarian cancer cell lines following blueberry extract treatment. It killed tumor cells at a comparatively low $IC_{50}$ when compared to fibroblasts (normal cells). Freeze-dried blueberry has 50% growth inhibition ($IC_{50}$) of 79.2 and 69 μg/mL in OVSAHO and OV2008 ovarian cancer cell lines, respectively.

Anti-proliferative activity of the freeze-dried raspberry (*Rubus ideaus*) extract was evaluated using three human cell lines, MCF7 (breast adenocarcinoma, HT-29 (colon adenocarcinoma), and MRC-5 (human fetal lung, ECACC 84101801).[41] The obtained results showed that the breast adenocarcinoma MCF7 cell line was very sensitive to the freeze-dried raspberry. The 50% growth inhibition ($IC_{50}$) of the raspberry extract was 395.07μg/mL. It can be suggested that the presence of a wide range of phenolic compounds and vitamin C in raspberry (*Rubus ideaus*) extract contributed to their cytotoxic activity.[42] This is in accordance with the current results, which show that MCF7 was the most sensitive cell line to freeze-dried blueberry that has $IC_{50}$ of 64 μg/mL, as presented in Table 1. There was no difference in sensitivity between OV2008 ($IC_{50=}$ 69 μg/mL) and MCF7 ($IC_{50}$ = 64 μg/mL) [Figure 7]. Air-dried sample at 60°C and 90°C are also effective. They inhibited the growth of tumor cells at lower $IC_{50}$ than normal cells [Figure 7B and 7C). On the MDA-MB-231 breast cancer cell line, air-dried samples at 60°C had an $IC_{50}$ of 88.0 μg/mL at 90 °C has $IC_{50}$ 89.0 μg/mL. Cell viability appeared too high. This could be due to the high temperature of incubation (37°C), which could have contributed to the rapid degradation of phytochemicals. These bioactive compounds are highly unstable and may lose their activity when exposed to oxygen, light, and heat during processing, handling, and storage for an extended time. For instance, numerous studies showed that anthocyanins degrade in light, at high pH (higher than 7) and temperatures (higher than 60–80 °C) and in the presence of sulfite, ascorbic acid, and enzymes such as glycosidases, galactoside, peroxidizes, and phenolases.[43]However, this is a preliminary dataset, and further work is required to identify the active compounds and test their specific effects on cancer cell lines and the mouse model (*in vivo*). Recent studies have shown that berry extracts and their active compounds have the ability to inhibit cell proliferation, modulate cell cycle arrest, and induce apoptosis in cancer cells without affecting normal cells.[26] Another study has been evaluated the effects of three cultivars of blueberries ('Briteblue', 'Tifblue', and'Powderblue') on the liver HepG2 cancer cells.[27] The results showed that there were the greatest inhibitory effects of the blueberry anthocyanin fractions (ranging from 70 to 150 μg/mL concentrations), with 50% inhibition of cancer cell population growth.

Blueberries and blueberry extract positively affect biomarkers of genomic stability and indicators of carcinogenesis both *in vitro* and *in vivo*.[26] However, one major disadvantage of studying whole food or food extracts is that it is impossible to establish which particular dietary components (e.g. folic acid, flavonols, vitamin C, anthocyanins, and stilbenes) are the effective cytoprotective agents.

## Conclusion

The present study provided evidence that *Sidreoxelon mascatense fruits* exhibited anti-oxidant and anti-tumor activities due to the expected range of phytochemicals. Chromatographic analysis of these compounds is essentials to identify the active compounds under different drying methods, and these are very important quality of the industry product. The fruit extract with concentration (20 mg/mL) provided the maximum amount of vitamin C. Freeze-dried berries exhibit the highest % DPPH free radical scavenging activity from both flesh (50.76%) and seeds (39.37%) at concentration 20 mg/mL. However, there were no significant differences at various concentrations of blueberry extracts in both flesh and seed on their ability to scavenge the radical cation (ABTS+). The 50% growth inhibition ($IC_{50}$) of the freeze-dried blueberry extract at both cell lines was 64 and 69 μg/mL, respectively. Further

*in vivo* analysis is going to be relevant to elucidate the dual role of bleu berry extracts as anti-oxidant and anti-tumor.

In conclusion, our study found that phenolic compounds in blueberry fruit extract could inhibit the proliferation of the examined cancer cell lines. Further studies are required to clarify the mechanisms and to evaluate the bioavailability and metabolism of phenolic compounds in relation to reducing breast cancer risk.

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## Conflicts of Interest

There are no conflicts of interest.

## References


1. Ghazanfar SA. Handbook of Arabian medicinal plants: CRC press. 1994.
2. Amin A, Mousa M. Merits of anti-cancer plants from the Arabian Gulf region. Cancer Ther 2007;5:55-66.
3. Said SA, Al-Saadi SHA, Al-Abri AR, Akhtar MS, Weli AM, Al-Riyami Q. Cytotoxic properties of some herbal plants in Oman. J Taibah Univ Sci. 2014;2:71-74. doi:org/10.1016/j.jtusci.2014.01.004
4. Al-Attabi Z, Al Mamri R, Aslam KAA. Antioxidant potential properties of three wild omani plants against hydrogen peroxide-induced oxidative stress. Can J Clin Nutr 2015;2:16-22. doi:org/10.14206/canad.j.clin.nutr.2015.02.03
5. Al-Qalhati IR. Anti-diabetic Effect of pteropyrum scoparium and oxalis corniculata and their mixture in rats. Sultanate of Oman. [Dissertation/master's thesis]. Sultan Qaboos University. Sultanate of Oman. 2015.
6. Singh V, Guizani N, Essa MM, Rahman MS, Selvaraj S. In vitro antioxidant activities of Ziziphus spina-christi Fruits (red date) grown in Oman. Biotech. 2012;209-2016. doi: 10.3923/biotech.2012.209.216
7. Al-Yahyai R, Al-Nabhani H. Botanical description and phenological cycles of Monotheca buxifolia. Processding of the XXVII International Horticultural Congress-IHC2006: International Symposium on Asian Plants with Unique Horticultural. 2006. 769. doi:10.17660/ActaHortic.2008.769.35
8. Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, Krewer G. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species. J Agric Food Chem 1998;7:2686-93.
9. Jimenez-Garcia SN, Guevara-Gonzalez RG, Miranda-Lopez R, Feregrino-Perez AA, Torres-Pacheco I, Vazquez-Cruz MA. Functional properties and quality characteristics of bioactive compounds in berries: biochemistry, biotechnology, and genomics. Food Res Int 2013;1:1195-207. doi: org/10.1016/j.foodres.2012.11.004
10. Schmidt BM, Erdman JW, Lila MA. Differential effects of blueberry proanthocyanidins on androgen sensitive and insensitive human prostate cancer cell lines. Cancer Letter 2006;231:240-6
11. Tsuda H, Kunitake H, Kawasaki-Takaki R, Nishiyama K, Yamasaki M, Komatsu H, Yukizaki C. Antioxidant activities and anti-cancer cell proliferation properties of Natsuhaze (Vaccinium oldhamii Miq.), Shashanbo (V. bracteatum Thunb.) and blueberry cultivars Plants 2013;2:57-71
12. Smith MAL, Marley KA, Seigler D, Singletary KW, Meline B. Bioactive properties of wild blueberry fruits. Journal of Food Science 2000;65:352-6
13. Debnath-Canning M, Unruh S, Vyas P, Daneshtalab N, Igamberdiev AU, Weber JT. Fruits and leaves from wild blueberry plants contain diverse polyphenols and decrease neuroinflammatory responses in microglia. Journal of Functional Foods 2020;68:103906
14. Seo HS, Adams SH, Howard LR, Brownmiller C, Hogan V, Chen JR, Pramudya RC. Children's liking and wanting of foods vary over multiple bites/sips of consumption: a case study of foods containing wild blueberry powder in the amounts targeted to deliver bioactive phytonutrients for children. Food Research International 2020;13:108981.
15. Li W, Hydamaka AW, Lowry L, Beta T. Comparison of antioxidant capacity and phenolic compounds of berries, chokecherry and seabuckthorn. Cent Eur J Biol 2009;4:499-506. doi: 10.2478/s11535-009-0041-1
16. Produce Report. An overview of global blueberry production in 2016. https://www.producereport.com-article-overview-global-blueberry-production-2016. Accessed 25 July 2020.
17. Whyte AR, Schafer G, Williams CM. Cognitive effects following acute wild blueberry supplementation in 7- to 10-year-old children. Eur J Nutr 2016;55:2151-62
18. Feng C, Su S, Wang L, Wu J, Tang Z, Xu Y, Wang L. Antioxidant capacities and anthocyanin characteristics of the black-red wild berries obtained in Northeast China. Food Chem 2016;204:150-8. doi:org/10.1016/j.foodchem.2016.02.122
19. Fu Y, Zhou X, Chen S, Sun Y, Shen Y, Ye X. Chemical composition and antioxidant activity of Chinese wild raspberry (Rubus hirsutus Thunb.). LWT-Food Sci Technol 2015;2:1262-8. doi:org/10.1016/j.lwt.2014.09.002
20. Brauch J, Buchweitz M, Schweiggert R, Carle R. Detailed analyses of fresh and dried maqui (Aristotelia chilensis (Mol.) Stuntz) berries and juice. Food Chem 2016;190:308-16. doi:org/10.1016/j.foodchem.2015.05.097
21. Folmer F, Basavaraju U, Jaspars M, Hold G, El-Omar E, Dicato M, Diederich M. Anticancer effects of bioactive berry compounds. Phytochem Rev 2014;1:295-322. doi: 10.1007/s11101-013-9319-z
22. Riso P, Klimis-Zacas D, Del Bo' C, Martini D, Campolo J, Vendrame S, Møller P, Loft S, De Maria R, Porrini M. Effect of a wild blueberry (Vaccinium angustifolium) drink intervention on markers of oxidative stress, inflammation and endothelial function in humans with cardiovascular risk factors. Eur J Nutr 2013;52:949-61
23. Duffy KB, Spangler EL, Devan BD, Guo Z, Bowker JL, Janas AM, Mouton PR. A blueberry-enriched diet provides cellular protection against oxidative stress and reduces a kainate-induced learning impairment in rats. ?Neurobiol Aging 2008;11:1680-9. doi:org/10.1016/j.neurobiolaging.2007.04.002
24. Stoner GD, Chen T, Kresty LA, Aziz RM, Reinemann T, Nines R. Protection against esophageal cancer in rodents with lyophilized berries: potential mechanisms. Nutr Cancer 2006;1:33-46. doi: 10.1207/s15327914nc5401_5
25. Misikangas M, Pajari AM, Päivärinta E, Oikarinen SI, Rajakangas J, Marttinen M, Mutanen M. Three Nordic berries inhibit intestinal tumorigenesis in multiple intestinal neoplasia/+ mice by modulating β-catenin signaling in the tumor and transcription in the mucosa. J Nutr 2007;10:2285-90. doi:org/10.1093/jn/137.10.2285
26. Duthie SJ. Berry phytochemicals, genomic stability and cancer: evidence for chemoprotection at several stages in the carcinogenic process. Mol Nutr Food Res 2007;6:665-74. doi:10.1002/mnfr.200600257
27. Seeram NP. Berry fruits for cancer prevention: current status and future prospects. J Agric Food Chem 2008;3:630-5.
28. Qi C, Li S, Jia Y, Wang L. Blueberry anthocyanins induce G2/M cell cycle arrest and apoptosis of oral cancer KB cells through down-regulation methylation of p53. Yi chuan= Hereditas/Zhongguo yi chuan xue hui bian ji. 2014.36(6):566-573
29. Lin W and LI Z. Blueberries inhibit cyclooxygenase-1 and cyclooxygenase-2 activity in human epithelial ovarian cancer. Oncology Letters. 2017;13:4897-4904
30. World Health Organization (WHO). https://www.who.int/cancer/prevention/diagnosis-screening/breast-cancer/en/ accessed 25 July 2020

31. Lee SN, Kang KJ. The effect of blueberry on ROS accumulation and cell death in human normal breast epithelial (MCF10A) and breast cancer (MCF7) cells. Korean J Food & Nutr 2008;21:416-24
32. Adams LS, Phung S, Yee N, Seeram NP, Li L, Chen S. Blueberry phytochemicals inhibit growth and metastatic potential of MDA-MB-231 breast cancer cells through modulation of the phosphatidylinositol 3-kinase pathway. Cancer Res 2010;70:3594-605
33. Davidson KT, Zhu Z, Balabanov D, Zhao L, Wakefield MR, Bai Q, Fang Y. Beyond conventional medicine – a look at blueberry, a cancer-fighting superfruit. Pathology & Oncology Research 2018;24:733-8
34. Waly MI, Al-Rawahi AS, Al Riyami M, Al-Kindi MA, Al-Issaei HK, Farooq SA, Rahman MS. Amelioration of azoxymethane induced-carcinogenesis by reducing oxidative stress in rat colon by natural extracts. BMC Complement Altern Med 2014:1-14. doi: 10.1186/1472-6882-14-60
35. Yemmireddy VK, Chinnan MS, Kerr WL, Hung YC. Effect of drying method on drying time and physico-chemical properties of dried rabbiteye blueberries. LWT – Food Sci Technol 2013;2:739-45. doi: org/10.1016/j.lwt.2012.07.011
36. Connor AM, Luby JJ, Hancock JF, Berkheimer S, Hanson EJ. Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage. J Agric Food Chem 2002;4:893-8. doi:org/10.1021/jf011212y
37. Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J. Bioactive compounds and antioxidant activity in different types of berries. Int J Mol Sci 2015;10:24673-06. doi:10.3390/ijms161024673
38. Stratil P, Klejdus B, Kubáň V. Determination of phenolic compounds and their antioxidant activity in fruits and cereals. Talanta 2007;4:1741-51. doi: org/10.1016/j.talanta.2006.08.012
39. Kwok B, Hu C, Durance T, Kitts D. Dehydration techniques affect phytochemical contents and free radical scavenging activities of Saskatoon berries (Amelanchier alnifolia Nutt.). J Food Sci 2004;3:122-6
40. Vulić JJ, Velićanski AS, etojević-Simin DD, Tumbas-Šaponjac VT, ilas SM, Cvetković DD, Markov SL. Antioxidant, antiproliferative and antimicrobial activity of freeze-dried raspberry. Acta Period Technol 2014;45:99-116. doi: 10.2298/APT1445099
41. Reque PM, Steffens RS, Silva AMD, Jablonski A, Flôres SH, Rios ADO, Jong EVD. Characterization of blueberry fruits (Vaccinium spp.) and derived products. Food Sci Technol (Campinas) 2014;4:773-9. doi: org/10.1590/1678-457X. 6470
42. Da Rocha Concenço FIG, Stringheta PC, Ramos AM, Oliveira IHT. Blueberry: functional traits and obtention of bioactive compounds. Am J Plant Sci 2014;18:2633. doi:org/10.4236/ajps.2014.518278
43. Rodríguez K, Ah-Hen KS, Vega-Gálvez A, Vásquez V, Quispe-Fuentes I, Rojas P, Lemus-Mondaca R. Changes in bioactive components and antioxidant capacity of maqui, Aristotelia chilensis [Mol] Stuntz, berries during drying. LWT-Food Sci Technol 2016;65:537-42. doi: org/10.1016/j.lwt.2015.08.050