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Phenolics and Flavonoid Contents of Freeze-dried Vaccinium myrtoides (Blume) Miq. Contribute to Decreased Reactive Oxygen Species (ROS)

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ABSTRACT

Vaccinium species (Family Ericaceae) include the blueberries, with its fruits widely studied for its antioxidant and reactive oxygen species (ROS) scavenging activity. However, *Vaccinium myrtoides* Blume (Miq.) is a species native to the Philippines that has not yet been thoroughly examined for its health-boosting properties. This study delivers new information on *Vaccinium myrtoides* fruit extract regarding its antioxidant and ROS scavenging activity as applied to *in vitro* and *in vivo* animal models. Crude ethanolic extracts of ripe freeze-dried *V. myrtoides* fruits (BBE) resulted to a significant increase in total phenolics and total flavonoid contents, with its highest concentration observed at 1:100 dilution. Antioxidant analysis of BBE was significantly highest at 1:50 dilution using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity test. *In vitro* administration of BBE extract at 50 and 200 µg mL^{-1} to phorbol 12-myristate-13-acetate (PMA)-stimulated murine macrophages resulted to 100 times lower ROS production, while *in vivo* administration of 100 and 400 mg kg^{-1} BBE via gavage to sexually mature male ICR mice immunosuppressed with cyclophosphamide at 150 mg kg^{-1} for two weeks drastically reduced the amount of oxidative stress produced from cyclophosphamide and PMA administration. The present findings highly suggest increased antioxidant and ROS scavenging activities upon addition of *V. myrtoides* ripe fruit extract, which can be alluded to its phenolic and flavonoid contents.

Key words: antioxidant, blueberry, ROS scavenging, total flavonoids, total phenolics, *Vaccinium myrtoides*
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INTRODUCTION

The *Vaccinium* genus (Family Ericaceae) are deciduous shrub plants composed of either shrubs or small, rarely medium-sized trees, terrestrial or epiphytic. Prominent members of this family include blueberries and cranberries, both known for its health-promoting polyphenolic compounds. Blueberries are considered as one of the five major healthy fruits by the Food and Agriculture Organization (FAO) (Sun et al. 2015) brought about by its antioxidant and reactive oxygen scavenging (ROS) activities influenced by its polyphenolic composition, including phenolics, flavonoids, and anthocyanins. Hence, there has been an upsurge in the use and incorporation of berries in products for everyday consumption. Its health-promoting properties are influenced by several factors: species, genotype, age and developmental stage, ripening stage, climatic factors, agronomic management, and postharvest conditions (Yuan et al. 2011; Peña-Sanhueza et al. 2017; Sun et al. 2018).

Postharvest conditions, especially its storage, can also affect the phytochemical content. Freeze-dried foods are classified under the dried, desiccated, or low-moisture foods that do not contain more than 25% moisture (Reque et al. 2014; Jay et al. 2005). Although fresh frozen berries and juices can retain its antioxidant capacity similar to newly harvested ones until three months of storage, this begins to decrease by six months possibly due to postharvest reactions concerning phenolic metabolism as mediated by polyphenol oxidase (PPO) (Reque et al. 2014; Lohachoompol et al. 2004). Freeze-drying can retain better total phenolics and antioxidant capacity compared to other preparations (e.g. hot-air drying, microwave-drying), although the ascorbic acid content is lower in freeze-dried plant material (Reyes et al. 2011; Mejia-Meza et al. 2008).

Although its temperate cousins are well-studied, *Vaccinium myrtoides* is an underutilized fruit in the Philippines (Coronel 2011). It is a native blueberry mainly distributed in the Northern Celebes, Peak of Tidore in Moluccas, North Maluku and Sulawesi in Indonesia (Argent 2008), and Luzon and Mindanao in the Philippines (Coronel 2011; van Steenis 1967; Merrill 1926). It appears in two ethnobotanical surveys (Balangcod and Balangcod 2009; Chua-Barcelo 2014) as a wild fruit that serves as a good source of food and economic products.

Only two studies have examined the phytochemical and antioxidant contents of *V. myrtoides* fruits. Barcelo (2015) profiled the phytochemical composition of fresh fruits (alkaloids, sterols, tannins, and high amounts of flavonoids and polyphenols), while Barcelo et al. (2015) examined the antioxidant activity of methanolic extracts of fresh blueberry fruits, resulting to 80.02% radical scavenging activity using the DPPH assay. Apart from the fruits, Galvez (2015) demonstrated that the ethanolic extract of *V. myrtoides* leaves also contain phytosterols, flavonoids, phenolics and tannins, and have high antioxidant activity with an IC_{50} of 20.85 µg mL^{-1}, compared to that of ascorbic acid at 21.56 µg mL^{-1}. Partial antibacterial activity against *Pseudomonas aeruginosa* was also observed in blueberry leaves (Galvez 2016). The aforementioned results about *V. myrtoides* fruit and leaves were also reiterated by Mirghani et al. (2019) as this species was included in their discussion reiterating the importance and health benefits of various wild *Vaccinium* species.

These studies show that the fruits and leaves of *V. myrtoides* contain considerable amounts of polyphenolic compounds and have potent antioxidant activity. Though both plant organs have been quantified for phytochemicals and antioxidant activity, the fruit would be best to use as these are the ones consumed by the public for its health-promoting qualities.

These findings suggest that *V. myrtoides* possesses compounds that could aid in reducing the damages from oxidative stress, as an abundance of ROS can weaken the immune system and could contribute to the onset of various diseases. Although other *Vaccinium* species have had numerous studies of its fresh and preserved fruits regarding its antioxidant and ROS scavenging activity when applied to *in vitro* and *in vivo* experiments, there has been no record of such for *V. myrtoides*. Hence, this is the first known study that aims to investigate the effectivity of *V. myrtoides* ripe fruit crude ethanolic extract as an antioxidant and its ROS scavenging activity in *in vitro* and *in vivo* experiments using immunosuppressed male ICR mice.

MATERIAL AND METHODS

All procedures conducted in mice were approved by the University of the Philippines Los Baños Animal Care and Use Committee with approval number AR-2018-258.

Authentication of Plant Samples

Vaccinium myrtoides fruiting branch was submitted to the Museum of Natural History Botanical Herbarium, University of the Philippines Los Baños (CAHUP) for authentication by examining following features such as leaf morphology, apical racemic inflorescence with pink to cream-colored florets, and bluish-blackish, globose fruit with diameter around 4-5 mm (van Steenis 1967; Merrill 1926).

Vaccinium myrtoides Fruit Extract (BBE) Preparation and Extraction

Ripe reddish to dark blue or violet color *V. myrtoides* fruits were harvested from a farm in Benguet, Northern Philippines. Berries were lyophilized using a Labconco® FreeZone 2.5 Liter Benchtop Freeze Dry System (Kansas City, Labconco Corporation), ground using a Wiley® Mini-Mill grinder (sieve 20) twice (New Jersey, Thomas Scientific®), and stored in an amber bottle inside the refrigerator until use. Ethanolic extraction of blueberry fruit was adapted from Grace et al. (2014) with slight modification. In brief, powdered freeze-dried berries (2.5 g x 3

replicates) ground with 250 mL 80% ethanol using mortar and pestle, and suction filtered using a Büchner funnel through Whatman No. 1 filter paper (Darmstadt, Merck®) until colorless extract was achieved. Filtrates for each replicate were pooled and concentrated through rotary evaporation at 40°C using Büchi Rotavapor® R-114/S (Flawil, Büchi Labortechnik AG). Concentrated *V. myrtoides* appeared sticky, viscous, and did not dry easily.

Phytochemical Analysis

BBE extracts were diluted at 1:10, 1:50, and 1:100 using ethanol for phytochemical assays (Folin-Ciocalteu and aluminum chloride). Methods for both were adapted from Barcelo et al. (2015) and Ebrahimzadeh et al. (2008) with modifications.

Folin-Ciocalteu assay for total phenolic content

Samples of 1 g of 10 µL BBE were added with 35 µL of distilled water and 10 µL of Folin-Ciocalteu reagent, mixed, and allowed to stand for five minutes for reaction to take place. The mixture was neutralized with 45 µL of 7 % $NaHCO_3$ and kept in the dark at room temperature for 90 minutes. The absorbance was measured at 765 nm using a ThermoScientific® MultiSkanGo plate reader (Ratastie 2, Thermo Fischer Scientific) where 80% ethanol served as the blank. Gallic acid standards ranging from 0.05 to 0.9 g mL^{-1}were prepared in 80% ethanol from a 1 g mL^{-1}stock concentration. Obtained results were expressed as milligrams gallic acid equivalent (GAE) per 100 mg fresh weight (fw). All values expressed were the means of four replicates.

Aluminum chloride assay for total flavonoids

Ten microliters of BBE was added with 30 µL 80% ethanol, 5 µL of 10% aluminum chloride, 5 µL of 1M potassium acetate, and 50 µL of deionized distilled water, mixed, and left to stand in the dark at room temperature for 30 minutes for the reaction to take place. Absorbance was measured at 415 nm (ThermoScientific® MultiSkanGo plate reader). Standards from 0.05 to 0.9 g mL^{-1} were prepared from quercetin in 80% methanol in 1 g mL^{-1} stock concentration. Total flavonoids were expressed in mean milligrams of quercetin equivalents (QE) per 100 mg of fresh weight (fw). All values expressed were the means of four replicates.

Assay for Bioactivities

Antioxidant Activity Through 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

The same dilution of BBE (1:10, 1:50, and 1:100) was used for the DPPH assay. This was adapted from Barcelo et al. (2015) and Ebrahimzadeh et al. (2008) with modifications. In brief, 10 µL of BBE from the different dilutions were added with 190 µL of DPPH from a 24 mg/L stock solution in 80% methanol prepared in an aluminum foil-wrapped test tube. The mixture was shaken for a minute then left to stand in the dark at room temperature for 30 minutes for reaction to take place. Absorbance (Abs) was read at 517 nm (ThermoScientific® MultiSkanGo plate reader) with 80% methanol as blank and an 80% methanol with DPPH as negative control. Assay was carried out in quadruplicate and the mean values were obtained. The DPPH radical scavenging activity (%) was computed using the following formula:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \left(\frac{Abs_{negative\ control} - Abs_{sample}}{Abs_{negative\ control}}\right) \times 100$$

***In vitro* Intracellular Reactive Oxygen Species (ROS) Production Assay**

Macrophage and splenocytes were sourced from both peritoneal lavage and mashed spleens from ten 8-week old male ICR mice obtained from the Laboratory Animal Facility of the Research Institute for Tropical Medicine (RITM), Muntinlupa City, Philippines. This assay uses unstimulated and phorbol 12-myristate-13-acetate (PMA)-stimulated cells to compute for the produced ROS.

Reaction mixtures were added after the cells were incubated in the wells for three hours. Another hour of incubation followed suit. The protocol from Nudo and Catap (2011) for extracellular superoxide anion production was done accordingly. The macrophage concentration was adjusted to 6 X 10^6 cells mL^{-1} and 100 µL aliquots were added to wells. The plate was incubated at 30 °C humidified environment for 90 minutes to allow macrophage attachment. Four tubes were prepared for each replicate sample: tubes 1 and 2 were used to measure unstimulated macrophages for intracellular ROS production, while tubes 3 and 4 contained PMA-stimulated macrophages to measure stimulated ROS production. For tubes 1 and 2, 200 µL of superoxide dismutase (SOD) with a final concentration of 30 µg mL^{-1} was added while tubes 3 and 4 received 100 µL of PMA with a final concentration of 0.5 µg mL^{-1}.

Another set of tubes with unstimulated macrophages were added with 5 µg mL^{-1}, 50 µg mL^{-1}, and 200 µg mL^{-1} of BBE and incubated for 90 minutes. All tubes contained 1.6 mL of nitroblue tetrazolium (NBT) with a final concentration of 0.8 mg mL^{-1}. Blanks were prepared containing the above mentioned BBE concentrations per tube without the macrophages. After the incubation of macrophages, supernatant was removed, and macrophages were washed with warm (30 °C) Hank's Balanced Salt Solution (HBSS). Reaction mixtures, composed of either SOD and NBT or PMA and NBT, of 100 µL volume prepared earlier were added to triplicate wells. The plates were incubated again at 30 °C for an hour then the macrophages were washed thrice with 70% methanol and air dried at room temperature. Aliquots of 120 µL 2 M KOH and 140 µL DMSO was added to each well and mixed thoroughly. Absorbance was read at 630 nm (ThermoScientific® MultiSkanGo plate reader) after a minute of shaking.

The NBT dye, when oxidized, precipitates at the site of reaction as dark blue granules. It is usually employed in *in vitro* assays to evaluate the amount of ROS produced (Fattorossi et al. 1990). The rate of

superoxide anion radical production at a single time point was used to make comparisons between different exposure groups. Change in absorbance was calculated using the equation below:

$$ROS\ Concentration = \left(\frac{No\ SOD\ absorbance - Blank}{SOD\ absorbance - Blank}\right) \times 15.87$$

Where:
No SOD: mean absorbance of the non-SOD-containing wells
SOD: mean absorbance of the SOD-containing wells

By multiplying the change in absorbance by 15.87, the nanomole (nmol) concentration of intracellular SOD-inhibitable superoxide anion radical was computed. Data were expressed as nmol $O_2/2 \times 10^5$ cells unit time^{-1}.

In vivo Antioxidant and ROS Scavenging Assays

Thirty six 10-week old male ICR mice purchased from the RITM, Muntinlupa City, Philippines, were individually caged in standard polycarbonate cages with stainless steel top and maintained at 22±2 °C, 30-70% humidity and 12H:12H light:dark cycle with *ad libitum* feeding with mouse commercial pellets (Altromin®, Germany) and distilled water.

After the one week acclimation period, mice were randomly allocated into four (4) groups: Group 1, given 1% body weight (BW) phosphate buffer solution (PBS) pH 7.0; Group 2, given 150mg kg^{-1} BW cyclophosphamide (Xyclomed®, Korean United Pharma) and served as the positive control; Group 3, given 100 mg kg^{-1} BW BBE extract + cyclophosphamide; and Group 4, given 400 mg kg^{-1} BW BBE + cyclophosphamide. BBE extracts were prepared by reconstituting the 250 mg mL^{-1} stock concentration of BBE with PBS and then sterilized using 25 mm nylon wielded syringe filters (0.22 µm, China, LabFil®) done under a biosafety cabinet. Vehicle and BBE extracts were administered once a day for two weeks using a stainless-steel gavage, while the cyclophosphamide was injected intraperitoneally one hour after BBE extract administration once a day every three days for two weeks. Mice were euthanized through cervical dislocation. A midventral abdominal incision was made to exteriorize the spleen for macrophage and splenic harvesting following the protocol of Catap et al. (2018).

Statistical Analysis

All data were presented as mean SD and analyzed using SAS version 9.4. Total phenolic content, total flavonoid content, and percentage radical scavenging activity among the different treatment groups were analyzed using Friedman's Chi Square Test at P<0.05, while ROS scavenging activity was analyzed using Least Significant Difference (LSD) at P<0.05.

RESULTS

Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Antioxidant Activity Through 2,2-diphenyl-1-picrylhydrazyl (DPPH) of *V. myrtoides* BBE

Mean TPC, TFC, and antioxidant activity per dilution of freeze-dried BBE extract is shown in Table 1. TPC and TFC significantly increased with increasing dilutions of the BBE extract with highest amount observed at 1:100 dilution of BBE. However, it was observed that the TFC was proportionally increasing with the dilutions. On the other hand, significantly high antioxidant activity using DPPH assay was achieved using the 1:50 BBE extract.

Table 1. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Percentage Radical Scavenging Activity of BBE in three dilutions.

BBE sample dilution	Phytochemical Assays		Antioxidant Activity
	TPC (mg GAE 100 mg fresh weight^{-1})	TFC (mg QE/ 100 mg fresh weight^{-1})	% DPPH radical scavenging activity
1:10	184.29 ± 43.22^{c}	124.60 ± 13.83^{c}	47.70± 30.62^{a}
1:50	256.44 ± 42.25^{b}	253.41 ± 24.36^{b}	63.24 ± 11.60^{b}
1:100	314.05 ± 49.39^{a}	408.44 ± 35.67^{a}	39.37 ± 4.76^{c}

Note: Means followed by different letters in the same column are significantly different (Friedman's Chi Square Test; P<0.05).

In vitro ROS Scavenging Activity

It is significant that the presence of 50 µg mL^{-1} and 200 µg mL^{-1} BBE extract *in vitro* registered low ROS production comparable to the state of unstimulated murine macrophages. This state is 100 times lower than that of the PMA-stimulated macrophages (Table 2). This means BBE has high ROS scavenging activity. However, it is noticeable that the 50 and 200 µg mL^{-1} had the same effect, which could imply that the BBE effect has a threshold value. With the two BBE concentrations not showing significant difference in effect may mean that a threshold concentration value of the substance, when reached, is enough to register appreciable ROS scavenging activity. The activity is not linearly correlated with substance concentration. Nevertheless, these infer that BBE extract has immunomodulatory activities through reduction of ROS concentration at 50 µg mL^{-1} and 200 µg mL^{-1} through ROS scavenging.

Table 2. Mean ROS Concentration *in vitro* with BBE at various concentrations.

Treatment Groups	Mean
Unstimulated macrophages	-0.07^{c} ± 0.158
Macrophages + PMA	0.07^{ab} ± 0.087
Macrophages + 5 µg mL^{-1} BBE	0.10^{a} ± 0.064
Macrophages + 50 µg mL^{-1} BBE	-0.06^{c} ± 0.064
Macrophages + 200 µg mL^{-1} BBE	-0.06^{c} ± 0.064

Note: Means followed by different letters in the same column are significantly different (Least Significant Difference; P<0.05)

***In vivo* ROS Scavenging Activity**

Cyclophosphamide, a potent immunosuppressive drug, is known to induce oxidative cell damage via increased ROS production (Shokrzadeh et al. 2014) in the ICR treated male mice (Table 3). Treatment with BBE using 100 mg kg^{-1} or 400 mg kg^{-1} remarkably reduced ROS production in the cyclophosphamide treated mice (Table 3).

Table 3. Mean ROS Production of *in vivo* administration of BBE in two concentrations in unstimulated macrophages.

Treatment Groups	Mean
PBS (1% BW)	0.02 ± 0.090
Cyclophosphamide (150 mg kg^{-1})	0.14 ± 0.193
BBE (100 mg kg^{-1}) + Cyclophosphamide	-0.04 ± 0.284
BBE (400 mg kg^{-1}) + Cyclophosphamide	-0.13 ± 0.835

This suggests that both concentrations of BBE extract possess ROS scavenging activity. PMA and/or cyclophosphamide stimulation consistently resulted in an increase in ROS production in harvested macrophages from treated ICR mice (Table 4).

Table 4. Mean ROS Production of *in vivo* administration of BBE in two concentrations in PMA-stimulated macrophages.

Treatment Groups	Mean
PBS (1% BW) + PMA	0.07 ± 0.130
Cyclophosphamide (150 mg kg^{-1}) + PMA	0.12 ± 0.295
BBE (100 mg kg^{-1}) + Cyclophosphamide + PMA	-0.39 ± 0.338
BBE (400 mg kg^{-1}) + Cyclophosphamide + PMA	-0.14 ± 0.562

Treatment of male mice with BBE extracts at 100 mg kg^{-1} or 400 mg kg^{-1} markedly reduced ROS production in harvested macrophages. These findings reveal that BBE attenuated PMA and or cyclophosphamide-induced increase in ROS activity.

DISCUSSION

The present findings reveal that the BBE extract contained high amounts of phenolics and flavonoids, yet showed highest radical scavenging activity at 1:50 dilution. This could be attributed to the absorptivity of the solution and the limitation of the spectrophotometer. The BBE extract also showed remarkable reduction of ROS production and increased antioxidant activity in mice, whether administered to murine macrophages *in vitro* using 50 µg mL^{-1} and 200 µg mL^{-1} or to two-week cyclophosphamide-immunosuppressed mice *in vivo* at 100 mg kg^{-1} and 400 mg kg^{-1}.

The phytochemical and antioxidant activity of *V. myrtoides* fruits have been examined in two separate studies by the same author. Barcelo alone (2015) and with her colleagues (Barcelo et al. 2015) measured the TPC, TFC, and DPPH percent radical scavenging activity of methanolic extract of *V. myrtoides* fresh fruit which yielded 59.12 mg GAE/100 g fw, 1186.44 mg QE/100 g fw, and 80.02% radical scavenging activity, respectively. Comparing this to the TPC (314.05 mg GAE/100 mg fw) and TFC (408.44 mg QE/100 mg fw) of BBE extract at 1:100 dilution, the BBE extract resulted to higher polyphenolic and flavonoid content. However, the BBE extract in 1:50 dilution (63.24%) had a lower percentage radical scavenging activity compared to the results of Barcelo et al. (2015). Since it is readily available in the local markets in Benguet, *V. myrtoides* can be a candidate for a local fruit that can help satisfy the increased interest in diets containing berries and their fruit extracts, as there is a growing awareness in the health benefits of phenolic compounds.

In addition to this, an increase in the intake of fruits and berries may be associated with reduced incidences of disorders due to excessive ROS due to its protective effect from its phenolic compounds and antioxidant activity (Olas 2018). Results of the *in vitro* (Table 2) and *in vivo* (Tables 3 and 4) ROS scavenging assays show that the BBE extract is capable of reducing the oxidative damage induced either solely by PMA or a combination of cyclophosphamide and PMA administration. This suggests that the phytochemical constituents of the BBE extract could have contributed to the improvement of the antioxidant and ROS activity of the mice, as the medicinal effects of berries have been attributed to its high phenolic content, which include its specific groups the flavonoids and anthocyanins (Olas 2018; Huang et al. 2012).

The phenolics and flavonoids in the BBE extract determine its antioxidant and ROS scavenging activity, as shown in Table 1. They are capable of reacting with ROS and reactive nitrogen species (RNS) as its structures have an abundance of hydroxyls, resonance, and C=C bonds. Phenolic compounds are able to act as antioxidants by scavenging and suppressing radical species and upregulating and protecting existing antioxidant defenses. Its -OH groups can donate H^+ or e^- while its extended conjugated aromatic system can delocalize the unpaired electron (Skrovankova et al. 2015; Dai & Mumper (2010). It interferes with lipid peroxidation by donating H^+ to radicals, forming stable phenoxy radical intermediates ($PO^{\bullet}$) due to resonance and can react with other radicals to terminate the progression.

Since flavonoids are a subset of phenolics, flavonoids also possess an abundance of -OH groups suitable for antioxidant activity. With its general C6-C5-C6 structure based from chalcone, it exhibits features that add to its free-radical scavenging activity, such as the presence of 3',4'-dihydroxy structure in the B ring; the 2,3-double bond in conjunction with the 4-oxo group in the heterocycle, allowing conjugation between the A and B rings; and the presence of 3- and 5- hydroxyl groups in the A ring together with a 4-oxo function in the A and C rings. The extensive resonance of flavonoid structure makes it a highly effective antioxidant (Apak et al. 2016; Skrovankova

et al. 2015; Dai & Mumper 2010; González-Gallego et al. 2010; Zheng and Wang 2003).

The abundance of polyphenolic compounds in *Vaccinium* species allude its protective properties through its antioxidant activity against ROS. Most of the studies using different *Vaccinium* species report that it employs a combination of various effects, such as decreasing the oxidative stress commonly caused by formation of ROS and RNS usually from lipid oxidation and other cellular functions, decreasing amounts or expression of pro-inflammatory cytokines, i.e. interleukin 1 (IL-1), IL-6, and TNF-α, enzymes such as cyclooxygenase-2/COX-2, iNOS, myeloperoxidase (MPO), transcription factors and proteins like NF-κB, prostaglandin E2 (PGE2), and upregulating the expression or activity of in-house antioxidant enzymes, i.e. superoxide dismutase (SOD), catalase, and glutathione, in order to boost the immunity of the organism.

Several studies have shown these effects, whether done through *in vitro* or *in vivo* models. Ahmet et al. (2009) demonstrated the cardioprotective property in rats of a blueberry-enriched diet through mitigating ROS damage. Seneviranthne et al. (2010) worked with *V. corymbosum* hydrolysates abundant in phenolics reduced the H_2O_2-induced oxidative damages by significantly decreasing intracellular ROS generation through ROS scavenging. Hurst et al. (2010) noted that extracts from *V. corymbosum* applied to muscle myotubes from rats protected the cells from oxidative damage produced from exercise stress. Blueberries also exhibit hepatoprotection from cadmium- or CC14-induced hepatotoxicity through upregulation of expression of the antioxidant mechanisms in mice and amelioration of cellular injury and death caused by oxidative stress (Gong et al. 2014; Chen 2011). Tsuda et al. (2013) noted the inhibition of tumor growth in HL-60 human leukemia cells in vitro upon administration of blueberry extracts from *V. oldhamii* through its polyphenolic contents and antioxidant activity. Blueberries, along with other berry fruits, also have neuroprotective effects (Subash et al. 2014). Huang et al. (2016) noted the reduction of ROS and xanthine oxidase and upregulation of SOD and heme oygenase-1 due to malvidin and its glycosides from *V. ashei* extracts. Incorporation of local blueberries in the diet of rodents and humans, either as berry extracts or as whole blueberries, has also been suggested to help in lowering the risk of type 2 diabetes which has been linked with chronic activation of the inflammatory pathway (Stull 2016). Neuroprotective effects through antioxidant activity against ROS were better observed in *V. angustifolium* blueberries than in *V. vitis-idaea* lingonberries due to its more extensive array of phenolic compounds (Kelly et al. 2018). Although these antioxidant activities are observed in its cousins, these lend to the fact that the *V. myrtoides* blueberries have shown similar antioxidant activity from the tests done through *in vitro* and *in vivo* models.

From the results gleaned from its phytochemical composition and radical scavenging activity, it is suggested that the BBE extract can aid in improving the immune status of an organism, as seen in PMA-stimulated murine macrophages and cyclophosphamide-immunosuppressed mice models, through its effective reduction of ROS production and promotion of antioxidant activity due to its phenolic and flavonoid contents.

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