COMPARATIVE BIOCHEMICAL ANALYSIS OF VARIOUS BERRIES COLLECTED FROM FOGO ISLAND AND CHEMICAL ANALYSIS OF LINGONBERRIES USING HPLC-UV/DAD MS TOF ANALYZER

By

Farhana Ahmed

A thesis submitted to the

School of Graduate Studies in partial fulfillment of

the requirements for the degree of

Master of Science in Pharmacy

Memorial University of Newfoundland and Labrador

St. John's, Newfoundland and Labrador

August 2023

Abstract

Research on berries has been carried out for the last several decades due to their significant health benefits. This thesis presents an experimental study of different berries collected from Fogo Island, an offshore island of the province of Newfoundland and Labrador. The objective of this study was to evaluate the biochemical activities of different species of berries and to determine the phenolic constituents in lingonberries. Four types of berry samples including blueberries, crowberries, bunchberries and lingonberries, both fruits and leaves, were collected during the autumn season for two consecutive years from different locations on Fogo Island. All the samples were extracted and made into a powder using liquid nitrogen followed by sonication with a solvent system and centrifugation. Assays including the DPPH free radical scavenging capacity, total phenolic, total anthocyanin, total flavonoid and total tannin content of the samples were performed. Samples with the highest antioxidant activity were chosen for HPLC analysis using a HPLC UV/DAD MS TOF system. Blueberries and lingonberries showed the most promising results regarding antioxidant activities. Leaves showed higher antioxidant activity than fruits. More than 25 organic compounds were identified from lingonberries using HPLC analysis. Based on the promising results, it can be concluded that Fogo Island can be a lucrative spot to grow berries on a commercial basis. The presence of these phenolic constituents can explain the effects of lingonberries on human health and lead to additional pharmacological studies with extracts or specific compounds found in these berries.

Acknowledgments

First and foremost, I would like to thank the almighty God for providing me the intellectual capability and good health throughout the period of the Master's study to carry out this research. Then I express my heartiest gratitude to my research supervisor, Dr. John Weber for his valued guidance, support, and motivation throughout the program. Without his support, this research work would not have been possible. Next, I would like to thank Dr. Andrei Igamberdiev for giving me full opportunity to conduct my research using his laboratory at the Department of Biology, MUN. I also would like to extend my gratitude to Dr. Poorva Vyas for supervising me while conducting biochemical analysis and calculations. I would like to render my gratitude to other graduate students in the School of Pharmacy, Erin Kelly and Mathew Lamont, for supporting me during my time at Memorial. Also Dr. Shofiur Rahman, Post Doctorate researcher from the Department of Chemistry for guiding me and letting me use equipment in his laboratory during plant extraction for chemical analysis. I would also express my gratitude towards Dr. Stefana Egli of the Centre for Chemical Analysis, Research and Training of MUN for helping me with high-performance liquid chromatography analysis.

Above all, I owe my deepest thanks to my husband and my parents for their unconditional love, inspiration, and patience during my studies. Without my husband's longstanding inspiration and cordial support by accompanying me in Canada, it would be difficult for me to carry out the research smoothly.

Table of Contents

List of Tables

List of Figures

List of Symbols and Abbreviations

AA	Ascorbic acid
AD	Alzheimer's disease
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP-1	Activation protein-1
APX	Ascorbate peroxidase
BBB	Blood brain barrier
CAT	Catalase
CE	Catechin equivalents
COX	Cyclooxygenase
DAD	Diode array detector
dH_2O	Distilled water
DHAR	Dehydroascorbate reductase
DLB	Dementia with lewy bodies
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
ER	Endoplasmic reticulum
ESI	Electrospray ionization
GAE	Gallic acid equivalent
GPX	Guaiacol peroxidase
GR	Glutathione reductase
GSH	Glutathione
HIF-1α	Hypoxia-inducible Factor-1α
HPLC	High performance liquid chromatography
iNOS	Inducible nitric oxide synthase
LOX	Lipoxygenase
LPS	Lipopolysaccharide
MAPK	Mitogen activated protein kinase

MDHAR	Monodehydroascorbate reductase
MS	Mass spectroscopy
$NaNO_2$	Sodium nitrite
NDD	Neurodegenerative disorder
NF-κB	Nuclear factor kappa B
NMDA	N-methyl D-aspartate
PD	Parkinson's disease
PPAR-γ	Peroxisome proliferator-activated receptor gamma
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
R_t	Retention time
SE	Standard error
SOD	Superoxide dismutase
TAC	Total anthocyanin content
TFC	Total flavonoid content
TOF	Time of flight
TPC	Total phenolic content
TTC	Total tannin content
UPR	Unfolded protein response
UTI	Urinary tract infection
WHO	World health organization

Chapter 1: Introduction

1.1 General

We are now living in a world where scientists have potentially eradicated some diseases from the face of the earth forever using vaccines. On the other hand, there are still some diseases which are permanently incurable using conventional treatment procedures but manageable with proper diet and a healthy lifestyle. A healthy diet means consuming fruits and vegetables, which are inversely related to the incidence of several cancers, cardiovascular disease and age-related neurodegenerative disorders (Beattie *et al*., 2005).

The presence of antioxidants in a diet can decrease oxidative stress in the body which in turn minimizes the possibilities of dysfunction such as cancer, neurodegeneration and aging due to reactive oxygen species (ROS) (Demmig-Adams *et al*., 2002). In recent studies, berries have been found as an effective source of antioxidants and full of other beneficial nutraceuticals (Gundesli *et al*., 2019). Despite having a short growing season, Canada still grows berries and exports these to mitigate the world's needs. According to statistics, Canada is number two in exporting berries after the US. (Torabi, 2013). New technologies to overcome natural obstacles such as soil quality, climate and insects have been utilized by researchers in different provinces including Newfoundland and Labrador, in order to maximize the cultivation of berries (Debnath *et al*., 2016).

A wide spectrum of health benefits of berries makes them more interesting day by day to researchers (Halvorsen *et al*., 2002). Berries have been studied for their chemical composition and biological activities both *in vitro* and *in vivo*. (Juranić *et al*., 2008; Skrovankova *et al.,* 2015; Hossain *et al*., 2016). The quality of fruits is reported to vary depending on factors such as

location, climate, temperature and soil quality (Onwuka and Mang, 2018; Skrovankova *et al*., 2015). Considering these findings, different kinds of berries were collected from several areas of Fogo Island, NL for two consecutive years and compared based on their chemical and biological activity. In this study lingonberry samples from both years have been subjected to chemical investigation using high performance liquid chromatography (HPLC). A thorough qualitative and quantitative investigation has been performed to confirm the presence of various anthocyanins in lingonberry and determine the amount of major compound present in samples.

1.2 Fogo Island

Fogo island is the largest offshore island of Newfoundland and Labrador, located on the northeast coast of Newfoundland. This remote island is a living place of a few small communities (Gray, 2000). Tourists visit the Island for berry picking and fishing during nonwinter months, mostly July-August (Fogo Island by Robin Esrock; National Geographic). We chose Fogo Island as it's a popular destination for tourists for berry picking and locals celebrate the berry picking season here and often invite tourists to join them. In 2017, samples were collected from six locations which are Island Harbour, Lion's Den, Joe Batt's Pt. trail, Marconi site, Seldom and Tilting. In 2018 berries were collected from four locations which are Seldom, Island Harbour, Tilting and Simm's Beach of Fogo Island.

1.3 Lingonberries

Vaccinium vitis-idaea, commonly known as lingonberry, is an evergreen shrub. It is well known as partridgeberry in Newfoundland and red berry in Labrador, which is also the highest producer of these red fruits in the North American region. (Penney *et al.,* 1995). Although the Atlantic region has a short growing and cultivation period, picking berries and eating them while

they remain fresh is a celebration for the people who live in this area. (Opal Consulting Inc., 2010). Previously, lingonberries have been primarily collected from the wild but recently these plants have been domesticated (Padmanabhan *et al*., 2016). Now along with blueberries, lingonberries have become one of the most popular berry varieties of the *Vaccinium* species due to their link to preventing some chronic and degenerative diseases which is believed to be related to the presence of phenolic bioactive substances (Dróżdż *et al*., 2017).

1.4 Crowberries

Crowberries or *Empetrum nigrum* belong to the *Ericaceae* family (Boland., 2010). This species is known as blackberry in Newfoundland. Plants are low evergreen shrubs, which are native to subarctic, alpine and boreal regions. Fruits are juicy, edible and acidic in taste (Scott., 2010).

Crowberry was underutilized until recently when researchers discover its potential antioxidant activity (Jurikova *et al*., 2012). Due to this reason, it is also one potential crop in the list of "prospective super foods" (Seeram, 2008). Although fresh fruits are part of a traditional diet in North America, indigenous groups often consider fresh fruits as inedible as these contain a high amount of tannin which gives them an astringent and slightly acidic taste (Seeram, 2008, Laaksonen *et al*., 2011). However, fruits can be used in a mixture of jam or with drinks (Laaksonen *et al*., 2011). It is used in folk medicine in Korea as an anti-inflammatory agent to treat cystitis, nephritis, and urethritis (Park *et al*., 2012). Most health benefits are due to the presence of polyphenols, especially flavonols and anthocyanins (Ogawa *et al.,* 2008). Despite the potential utilization of crowberries, these have not been made commercially available yet

(Laaksonen *et al.,* 2011), but they might be a promising ingredient for the cosmetic and pharmaceutical industries (Hyun *et al*., 2016).

1.5 Blueberries

The highbush blueberry (*Vaccinium corymbosum*) and lowbush wild blueberry (*Vaccinium angustifolium*) are perennial flowering plants with blue or purple fruits (Rowland and Hammerschlag, 2005). There are two types of commercial blueberries, wild (lowbush) and cultivated (highbush), both of which are native to Northern America (Naumann, 1993). Blueberries (*V. angustifolium*) from the Island of Newfoundland, Canada are popular for their unique taste (Walther, 2018, Morton *et al*., 2018). One can collect them from almost anywhere on this Island in the months of August and September. They are the most popular berries due to their abundance, delicious taste and direct antioxidant action (Williamson and Clifford, 2010).

Blueberries are one of the richest fruits containing anthocyanins (Wu *et al*., 2006), which represent 60% of all polyphenols present in ripe fruits (Kalt *et al*., 2003). Statistics have shown that intake of approximately one-third of a cup of blueberries and anthocyanins 50 mg daily reduces the possibility of some diseases (Gonçalves *et al*., 2021; Cassidy *et al*., 2013; Cassidy *et al*., 2011; Jennings *et al*., 2012; McCullough *et al*., 2012). Also, magnetic resonance imaging of brain tissue showed that cognitive function in older persons has been greatly improved by drinking 9 ml/kg blueberry juice daily for up to 12 weeks (Krikorian *et al*., 2010). A study on school-aged children showed that it improves cognitive activities after taking a drink containing 30 g of blueberry powder (Whyte *et al*., 2016). Experiments on aging rats showed that ingesting 2% blueberries in a regular diet can protect vulnerable regions of the brain, mitigate inflammatory markers, and decrease oxidative stress (Poulose *et al*., 2014, Shukitt-Hale *et al*.,

2007 and Shukitt-Hale *et al*., 2019). The research on blueberries and all positive findings make it a promising fruit for neurodegenerative disorders and improving brain function.

1.6 Bunchberries

Bunchberry (*Cornus canadensis*) is a low herbaceous perennial plant (Gucker, 2012). It usually grows in cooler moist areas of Greenland, the east and west coasts of Canada, and Alaska (Hall *et al*., 1976). Fruits can be eaten raw or cooked (Turner *et al*., 1979). The taste is pleasant but without much flavor (Elias *et al*., 1982). Fruits can be added to breakfast cereal or can be used to make jam, pies and pudding (Facciola, 1990). Fruits can also be used with other low pectin fruits for making jam as they are high in pectin (Schofield, 1996), and pectin can provide some protection against radiation (Allardice, 1993).

For medicinal purposes, various parts of this plant also can be used. For example, leaves and stems have analgesic, cathartic and febrifuge properties (Moerman, 1998, Allardice, 1993). Plants can be used for pain relief, lung and kidney ailments, coughs and fever (Foster and Duke., 1990, Gahyur and Janssen, 2010). Also, a tea made from the roots can be used to treat infant colic (Foster and Duke., 1990). A concentrated liquid produced by boiling this plant can be used for eye wash (Foster and Duke., 1990. Moerman., 1998). Fruits are rich in pectin, hence it has carcinogenic properties and it is a capillary tonic, antioedemic, anti-inflammatory, antispasmodic and hypotensive (Duke and Ayensu., 1985).

1.7 Taxonomy of lingonberry

Lingonberry is native to the Northwest and Northeastern parts of Canada, Scandinavian countries, and some parts of the United States (Index of species information, USDA forest services). Internationally it is best known as lingonberry but the name varies according to

location such as lingen, lingon and puolukka in Finland, foxberries in Quebec, moss cranberry, mountain cranberry, northern mountain cranberry in other parts of Canada, partridgeberry in Newfoundland and red berry in Labrador (Penhallegon., 2006). Lingonberry usually grows in cooler parts of the world even in very harsh weather. Plants usually grow close to the ground which enables them to resist strong wind and also helps to trap moisture (Reich., 2004). This may be the reason why this plant is so popular with people who live in arctic regions. There are two types of lingonberry, which are wild berry and European berry. Wild berry is single blooming and only blooms during summer, while European berry blooms twice, once in late summer and then again in the Fall, from October to mid-November (Heidenreich., 2010).

1.8 Common uses and health benefits of lingonberries

In North America and Europe lingonberry is used as food to make jam, jellies, yogurt, cake, tart, ice cream or as a drink in liquor or tea (Ross *et al*., 2015). Lingonberries are also used by Indigenous Peoples in Canada where they eat them raw, stewed or with fish, meats and boiled eggs (Heller., 1953; Leighton., 1985). To treat frequent urination and diabetes lingonberries have been used as folkloric medicine by the Cree (Fraser *et al*., 2007; Leduc *et al*., 2006). Lingonberries are very rich in anthocyanins, which are responsible for blue, purple, red, orange and other colors and thereby these berries are often used as natural food colouring agents (Dróżdż *et al*., 2017). Berries can also be used as food preservatives as they contain high amounts of benzoic acid (Visti *et al*., 2003). Berries have been included in regular diets as fresh, frozen, or in processed forms (Manganaris *et al.,* 2014). The presence of phenolic compounds in berries makes them a good source of natural antioxidants (Samad *et al*., 2014; Paredes-López *et al*., 2010; Vyas *et al.,* 2013).

1.8.1 Antioxidant properties of lingonberries

Consuming natural dietary antioxidants is important as naturally occurring neutralizers or enzymes are not enough to combat free radicals present in the body and synthetic antioxidants potentially have harmful effects on the body (Lobo *et al*., 2010). Some research has shown that lingonberry has a higher antioxidant capacity than other berries (Wang & Jiao, 2000; Wang & Lin, 2000; Wang & Stretch, 2001). The reason for this may be that lingonberry contains a wide range of phenolic compounds including phenolic acids, tannins, anthocyanin and flavonoids (Wang *et al*., 2005). It also is a good source of pro-vitamin A (β-carotene), vitamin B (B1, B2 and B3), vitamin C, minerals (potassium, calcium, magnesium and phosphorus) and fibers (Oldemeyer & Seemel, 1976). Lingonberries have been shown to possess potent free radical scavenging activities against hydroxyl (OH-), di picryl hydroxyl (DPPH), peroxy (-ROO) and oxide (O^{2-}) free radicals, which in turn make these berries a very good source of natural antioxidants (Wang *et al*., 2005).

In the next section, how antioxidants work in living organisms is illustrated, showing their reducing properties and ability to reduce oxidative and nitrosative stress in the human body.

1.8.1.1 Reactive oxygen and nitrogen species

ROS are oxygen ions, peroxides, hydroxyls and the by-products of oxygen consuming metabolic processes in cells. They play an important role in regulating cellular signaling in proper concentrations. During stressed conditions, ROS concentration increases and they react with proteins, lipids, fatty acids in the body and can cause pathological changes or mutations (Morrell *et al*., 2008).

The human body has its own defense mechanisms against excess amounts of ROS (Held., 2015). There are number of enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and naturally occurring antioxidants such as ascorbic acid (AA), reduced glutathione (GSH), α-tocopherol, carotenoids, flavonoids, and the osmolyte proline which participate in detoxification process to further protect the body from mutation (Das *et al*., 2014).

Reactive nitrogen species (RNS) are the products of the reaction of nitric oxide with superoxide ions such as peroxynitrite and nitrogen dioxide, which play an important role in causing inflammation and other respiratory diseases (Ichinose *et al*., 1999). Like ROS, RNS play an important role in cellular signaling and can exert harmful effects when levels are elevated (Di Meo *et al*., 2016).

1.8.1.2 Oxidative and nitrosative stress

ROS and RNS play a dual role in cells. In lower concentrations they contribute to cell signaling pathways and in excess concentrations they are the cause of adverse changes in biomolecules such as DNA mutations, tumor proliferations and cell aging processes. Imbalances in the continuous process of developing and neutralizing of reactive species develop a stress in our body, which is called oxidative and nitrosative stress. (Winger *et al*., 2017).

1.8.1.3 Antioxidants and their roles in neutralizing ROS

Antioxidants are defined as stable enough molecules that can give an electron to a free radical to neutralize it and reduce the degradation of biomolecules by other reactive molecules

(Halliwell et al., 1995). Antioxidants exert their action by scavenging radicals, donating hydrogen or electrons, decomposing peroxide, quenching singlet oxygen, inhibiting enzymes and chelating with metals (Frie *et al*., 1988). They can eliminate reactive species in two proposed ways (Rice-Evans *et al*., 1993); One, by donating electrons to stabilize free radicals and second by chelating with the initiators of reactive species (Krinsky *et al*., 1992).

There are some natural antioxidants such as glutathione, ubiquinol, and uric acid (Shi *et al*., 1999) and a few enzymes in the body that can neutralize free radicals. There are also some non-enzymatic micronutrients which can promote free radical scavenging but cannot be produced by our body. Therefore, it is highly beneficial to consume foods that contain these micronutrients (Levine *et al*., 1999).

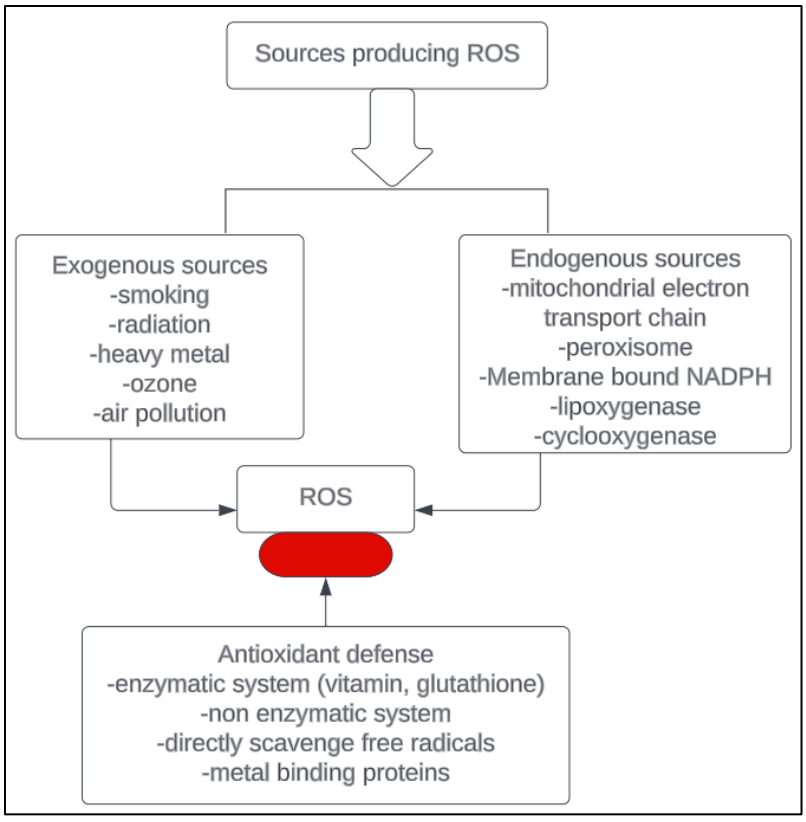

Figure 1.1: Schematic diagram of free radical production and antioxidants defense in body

(modified from Young & Woodside 2000, Sharifi-Rad *et al.,* 2020; drawn with Lucid chart)

Natural antioxidants include Vitamins A, C and E (Tafazoli *et al*., 2005, Vojdani *et al.,* 2000). Vitamin E (Figure1.2) is a lipid-soluble antioxidant which breaks the lipid peroxidation cycle by interfering with lipid peroxyl radicals (LOO˙) and terminating the lipid peroxidation chain reaction, which in turn produces tocopheroxyl radical, a comparatively stable compound and insufficiently reactive to initiate the cycle again (Witting *et al*., 1997, Morlière *et al*., 2012, Stocker *et al*., 1997).

$$LOO^{\cdot} + \alpha\text{-tocopherol–OH} \rightarrow LOOH + \alpha\text{-tocopherol–O}^{\cdot}$$

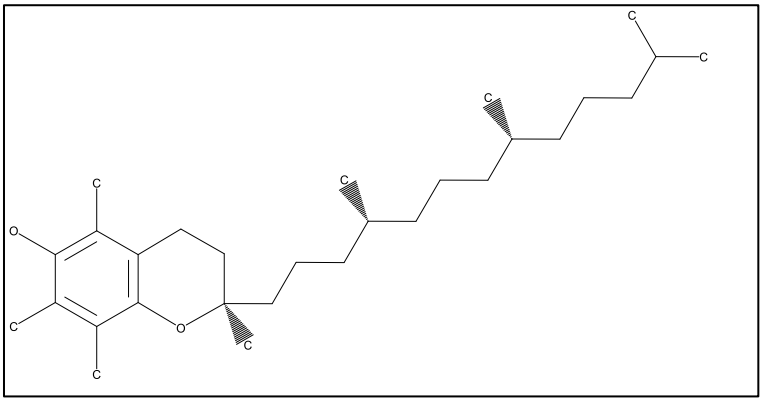

Figure 1.2: Structure of Vitamin E (drawn with Chem4word)

Vitamin C (Figure 1.3) is a water-soluble neutralizer which is capable of donating an electron to a lipid radical in order to terminate the lipid peroxidation chain reaction and convert to ascorbate radical itself (Niki *et al*., 1991, Retsky *et al*., 1991, Oh *et al*., 2010).

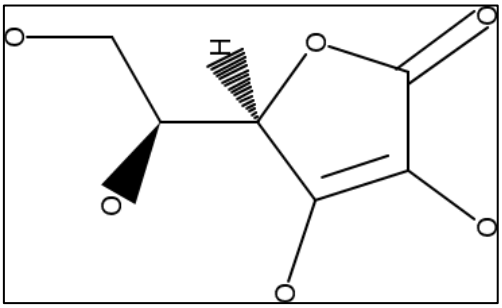

Figure 1.3: Structure of Vitamin C (drawn with Chem4word)

Vitamin A (Figure 1.4) is capable of protecting lipids against rancidity (Monaghan *et al*., 1932). This vitamin decreases low-density lipoprotein levels in the bloodstream, which in turn decreases the chance of heart disease (Parker *et al*., 1996, Vieira *et al*., 1995, Livrea *et al*., 1995, Tesoriere *et al.*, 1997). Bioflavonoids are another group of plant produced non-enzymatic antioxidants (Pietta *et al*., 2000, Nijveldt *et al*., 2001). They can inhibit hydroxyl radical induction DNA damage (Russo *et al*., 2000).

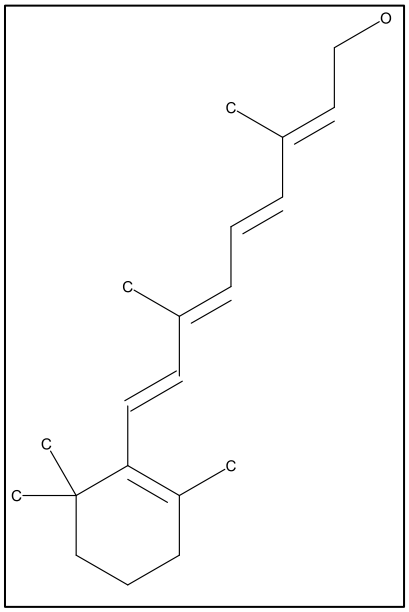

Figure 1.4: Structure of Vitamin A (drawn with Chem4word)

1.8.2 Anti-inflammatory effects of lingonberry

It is known that oxidative stress and inflammation are related. Oxidative stress can play a pathogenic role and can induce inflammation. ROS generated in brain tissue can modulate synaptic and non-synaptic transmission between neurons, which causes neuroinflammation, cell death and leads to neurodegeneration and memory loss (Popa-Wagner *et al*., 2013).

Inflammation is generally the unchecked response against a stimulation due to an endogenous signal or pathogens (Chen *et al*., 2018) and it is a defense mechanism which is vital for health (Nathan *et al*., 2010). Both infectious and non-infectious agents can trigger inflammatory cells and initiate inflammatory signaling pathways such as nuclear factor kappa B (NF-κB), mitogen activated protein kinase (MAPK), Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathways (Figure 1.5) (Chen *et al*., 2018). In acute inflammation, cellular and molecular interactions happen to minimize the severity of infection or damage. This mitigation process helps to restore homeostasis in the body but unchecked acute inflammation can lead to chronic inflammation (Zhou *et al*., 2016).

Polyphenols found in plants are able to neutralize free radicals and may reduce ROS-induced neuroinflammation. They also might modulate the activities of enzymes which regulate arachidonic acid metabolism (phospholipase A2), Cyclooxygenase (COX) and arginine metabolism (NOS). They may also modulate the production of some proinflammatory molecules (Hussain *et al*., 2016). A molecular mechanism of polyphenols to exert anti-inflammatory effects is inhibition of enzymes exerting proinflammatory properties such as COX-2, lipoxygenase (LOX), and iNOS, inhibition of NF-κB and activation of protein-1 (AP-1), phase-II antioxidant detoxifying enzymes, MAPK, protein kinase-C, and nuclear factor erythroid 2-related factor (Santangelo *et al*., 2007). AP-1, NF-κB, some enzymes and compounds (iNOS, COX-2), cytokines (IL-1β, TNF-α), neuropeptides, and proteases are known to be central in the process of inflammation (Kim *et al*., 2008).

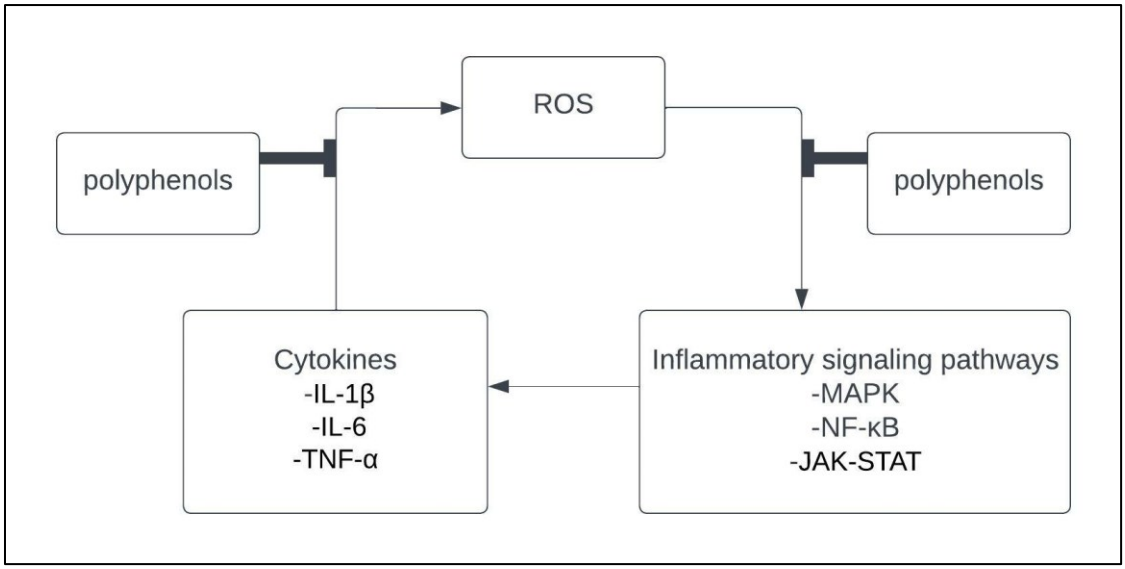

Figure 1.5: Schematic diagram of polyphenols inhibiting ROS induced signaling pathways (modified from Joseph *et al.,* 2014; drawn with Lucidchart).

Several studies have shown that proanthocyanin, flavonoids, polyphenolic acids, anthocyanins, and procyanidins are the main polyphenols present in lingonberry (Dróżdż *et al*., 2018). Another study determined the presence of procyanidins B1, B2, and A2 in lingonberry extracts, which has been shown to reduce oxidative status by reducing glutathione level in rats (Mane *et al*., 2011). Phenolic extracts have also been shown to successfully inhibit IL-6 and TNF-α production (Kylli *et al*., 2011. Hewage S. M *et al*., 2020). There is evidence that lingonberry fruit extract demonstrates anti-inflammatory actions in macrophage cell cultures by reducing the expression of pro-inflammatory mediators (IL-6, TNF-α, IL-1β, MCP-1, COX-2, iNOS). In the same study, the fruit extract also decreased ROS production in inflamed adipocytes by increasing expression of antioxidant defense enzymes (SOD, catalase, GPx) and inhibiting an oxidant enzyme (NADPH oxidase 4), which is one of the primary sources of ROS (Kowalska *et al*., 2020).

1.8.3 Anti-infection properties of lingonberry

Urinary tract infections (UTs) are one of the most common bacterial infections that occur mostly in women and comprise 25% of all infections. *E. coli* bacteria are the most common cause of UTIs (Al-Badr *et al*., 2013). Approximately 50%-60% of women report a UTI in their lifetime (Rahn *et al.,* 2008). One study showed that daily consumption of a mixture of lingonberry and cranberry juice reduces the reoccurrence of UTI whereas by comparison drinks containing lactobacilli were ineffective (Kontiokari *et al*., 2001).

Recurrences usually occur in people who use antimicrobial agents as prophylaxis for a long period. Using antibiotics for a long time might lead to resistance, which will then increase the need for alternative cures (Gupta *et al*., 1999, Reid *et al*., 2000). The bacteria responsible for UTIs usually arise from stools. Changes in one's regular diet can bring about a change in intestinal faecal bacteria (Gibson *et al*., 1998). Some research has shown that *Vaccinium* berries and products containing lactobacilli are effective against coliform bacteria that cause most of the UTI (Howe *et al*., 1998, Chan *et al*., 1985).

The berries of *Vaccinium* species contain the condensed tannin called proanthocyanidin, which can prevent the expression of P fimbriae of *E. coli* (Howell *et al*., 1998). Proanthocyanidins are a class of phenolic compounds and some of them have antimicrobial activity (Scalbert *et al*., 1991, Sen *et al*., 2001). These compounds demonstrate antibacterial activities by inhibiting cell wall synthesis and preventing bacterial adhesion to cell surfaces (Scalbert *et al*., 1991, Sen *et al*., 2001, Jones *et al*., 1994).

1.8.4 Anti-obesity properties of lingonberries and its effects on gut health

Obesity is now a worldwide health problem which is related to diet and lifestyle. Obesity causes other metabolic problems such as non-alcoholic fatty liver diseases, insulin resistance and dyslipidemia (Fabbrini *et al*., 2010). Accumulating evidence showed that low grade chronic inflammation is the main cause for these metabolic disorders (Hotamisligil *et al*., 2006). Gastrointestinal flora has an important role in controlling this subclinical inflammation (Ding *et al*., 2011). Gut composition plays an important role in maintaining body metabolism, immune responses and insulin sensitivity (Backhed *et al*., 2004, Vrieze *et al*., 2012, Cani *et al*., 2007, Ding *et al*., 2010, Cani *et al*., 2008). The metabolic endotoxemia and associated inflammation that are observed in obesity are the consequences of a dysfunctional gut barrier, which results in leakage of lipopolysaccharide (LPS) and proinflammatory cytokines into the circulation (Cani *et al*., 2007, Ding *et al*., 2010, Cani *et al*., 2008).

There is plenty of research that has shown that a fruit and vegetable-enriched healthy diet can reduce the risk of weight gain, type 2 diabetes, cardiovascular disease and stroke (Boeing *et al*., 2012). This might be due to the presence of polyphenols in vegetables and fruits such as berries (Wedick *et al*., 2012). One recent study showed that lingonberries can prevent high fat induced weight gain, liver weight, increased plasma levels of glucose, cholesterol and accumulation of body fat (Heyman-Lindén *et al*., 2016). The presence of reduced plasma levels of LPS in mice receiving high fat and lingonberries suggests that the presence of berries alters the gut microbes resulting in reduced leakage of proinflammatory LPS (Sun *et al.*, 2010). Polyphenols in berries can modulate the composition of gut flora by inhibiting the pathogenic microbes that in turn increase the number of beneficial bacteria (Lee *et al*., 2016). Gut permeability is controlled by a tight junction protein named occludin (Brun *et al*., 2007). An

increase in the enzyme occludin in mice receiving high fat and lingonberries indicate that polyphenols and proanthocyanidins in particular restore occludin, which reduces gut leakage of proinflammatory compounds and improves host health (Roopchand *et al*., 2015, Kim *et al.,* 2012, Pierre *et al*., 2013).

1.8.5 Anti-cancer properties of lingonberry

According to a World Health Organization (WHO) report, one of the major causes of morbidity and mortality worldwide is cancer (Stewart *et al*., 2014). The WHO report states that an unhealthy diet, being overweight and consuming low amounts of fruits and vegetables in the diet increase risk for developing cancer. *In vitro* and *in vivo* studies show that a diet emphasizing fruits and vegetables reduce the risk for developing cancer (Stewart *et al*., 2014, Boivin *et al*., 2007, Block *et al*., 1992, Steinmetz *et al*., 1991).

There are several proposed mechanisms or risk modulators for cancer such as oxidative stress, chronic inflammation, obesity, metabolic dysfunction, autophagy and apoptosis. These factors can contribute to, initiate, or propagate cancer (Kristo *et al.,* 2016). All of these factors together or individually shift the balance away from a healthy status of a body. Resveratrol and anethole compounds present in berries inhibit AP-1, which is related to growth regulation of genes involved in apoptosis and also accelerate the transition of epithelial cell to mesenchymal morphology, which is an early step marking metastasis. Resveratrol, a polyphenolic compound and flavopiridol, a semisynthetic flavonoid, have produced downregulation of the expression of apoptosis suppressor proteins (Bcl-2 and Bcl-XL) in a variety of cancer cell-lines (Aggarwal *et al*., 2006).

Many studies have shown that berries of the *Vaccinium* genus such as blueberries, lingonberries, cranberries and billberries positively influence gut microbial health, cardiac health and negatively promote tumor growth (Seeram *et al.,* 2014). In an experiment when blueberries and lingonberries were fed to mice the number and size of colonic adenoma was decreased, suggesting the tumor preventing capacities of those berries (Mutanen *et al*., 2008, Hoornstra *et al*., 2018).

1.8.6 Anti-aging properties of lingonberry

Lingonberries contain several anti-aging compounds, including vitamins A, C, and E, minerals, as well as other bioactive compounds which include proanthocyanidin, lignans, cyanidins and resveratrol (Stang *et al*., 1990). Resveratrol can increase life span of yeast and flies (Bhullar *et al*., 2015). *In vitro* studies have shown anti-inflammatory and anti-cancer properties of lingonberries, as well as positive effects against cardiovascular and Alzheimer disease (Bhullar *et al*., 2015). Studies show that lingonberry contains a higher amount of resveratrol than other *Vaccinium* species such as blueberry, billberry and cranberry (Maatta-Riihinen *et al*., 2002, Heininen *et al*., 2007). Proanthocyanidins present in lingonberries are short chained (dimer and trimer) which have higher bioavailability and potency than long chain proanthocyanidins (Maatta-Riihinen *et al*., 2002). Proanthocyanidin, anthocyanidin and resveratrol demonstrate anti-aging effects by scavenging free radicals and also by preventing wrinkles caused by ultraviolet radiation (Wang *et al*., 2005, Shen-Hong *et al*., 2000).

Collagen protein and elastin are important for skin health. Collagen structure maintains moderate cross linkage between proteins, but due to exposure to ultraviolet radiation or free radical oxidation, excessive cross linking can happen, which consequentially causes wrinkles.

However, antioxidants present in lingonberries can inhibit free radicals and protect skin from further damage. Also, studies showed that mice fed with lingonberries produced more hydroxyproline and SOD which are both beneficial for skin health (Valenzano *et al*., 2006, Baur *et al*., 2006., Lagouge *et al*., 2006, Kenjirou O *et al.,* 2013).

1.8.7 Protective effects of lingonberry in neurodegenerative disorders

According to recent studies, berry fruits are capable of preventing or slowing the progression of age-related neurodegenerative disorders (NDD) and improving motor and cognitive function (Subash *et al*., 2014). Many epidemiological studies suggest that flavonoid rich fruits can delay the symptoms of Parkinson's disease (PD), Alzheimer's disease (AD) and ischemic diseases (Ono *et al*., 2003; Savaskan *et al*., 2003; Marambaud *et al.*, 2005; Alzheimer's Association, 2008; Pandey and Rizvi, 2009). As oxidative stress and inflammation can trigger brain aging-related disorders (Casadesus *et al*., 2002), it can be assumed that antioxidants are beneficial for those diseases. *In vitro* studies and experiments on animals suggest that different antioxidants (*e.g.*, anthocyanin and caffeic acid) from berry fruits are effective for brain aging due to their anti-oxidative, anti-inflammatory, anti-viral and anti-proliferative properties (Youdim *et al*., 2001).

1.8.7.1 Brain aging caused by protein accumulation

Many factors can trigger neural disorders such as genetic factors, defective protein and fibre accumulation and prolonged oxidative stress. There are also several mechanisms that lead to neurodegeneration. One of the common theories is that misfolded protein accumulation in the endoplasmic reticulum (ER) leads to AD or PD and activates an unfolded protein response (UPR) (Haynes *et al*., 2004). An increase in misfolded protein increases oxidative stress (Frost and

Diamond, 2009; De Calignon *et al*., 2012; Hardy *et al.*, 2012). The UPR tries to normalize cell function, activating signaling pathways and if cell normalization does not take place by protein folding, ROS accumulation leads to cell death.

1.8.7.2 Parkinson's disease and lewy body dementia

PD is the second most common NDD and Dementia with Lewy bodies (DLB) is the second most common dementia after AD (Lees *et al*., 2009). The common pathological characteristic of both PD and DLB are the formation of Lewy Bodies (Lewandowsky, 1912; Okazaki *et al.*, 1961; Kosaka *et al*., 1976). Lewy Bodies are composed of alpha-synuclein and ubiquitin (Spillantini *et al*., 1997). Pathologically, aggregated alpha-synuclein proteins are seen in the Lewy bodies in various regions of the brain in PD (Spillantini *et al*., 1997). Folding and misfolding of this protein causes changes in the cell (Stefani and Dobson, 2003), such as accumulation of ROS and oxidative stress.

1.8.7.3 Glutamate excitotoxicity

Other than neurodegenerative and neurological disorders, there are other conditions which eventually result in production of reactive species. One such condition is excess activation of glutamate receptors, which is termed glutamate excitotoxicity (Weber, 2012). Glutamate is an excitatory neurotransmitter, which is essential for long-term potentiation, learning, memory, and other cognitive functions (Yu *et al*., 2008, Chen *et al*., 2009). When glutamate is released, it interacts with three classes of ionotropic glutaminergic receptors, namely N-methyl D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and Kainate receptors (Fig. 1.6) (Reidel *et al*., 2003). Activation of NMDA receptors leads to depolarization which, releases the Mg^{2+} block in the receptor and allows the transfer of Ca^{2+} ions (Spandou *et*

al., 2007). Activation of glutamate receptor for a long time, increases in the glutamate concentration at synapses, or decreases in the reuptake of glutamate lead to glutamate-mediated excitotoxicity (Lipton *et al*., 2008; Vincent and Mulle, 2009; Dong *et al*., 2009). Prolonged exposure to glutamate hyper-activates the cells and increases intracellular Ca^{2+} concentration, which activates a cascade of reactions that lead to cell lysis (Mehta *et al*., 2012). Cell lysis is a major contributing factor in various neuro-degenerative diseases and other disorders such as traumatic brain injury and stroke (Mehta *et al*., 2012).

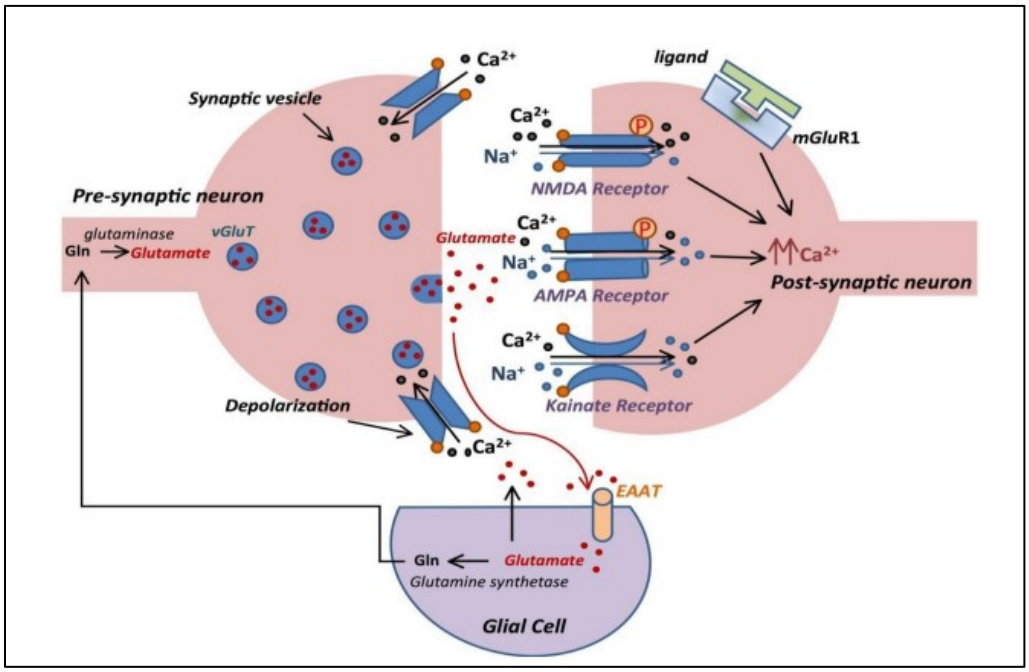

Figure 1.6: Glutamate-mediated neurotransmission and excitotoxicity

(modified from Weber, 2004).

Excessive intracellular Ca^{2+} activates different pathways which lead to cell death. Some enzymes such as endonucleases, proteases, phospholipase A2, and NO synthase are involved in these pathways (Figure 1.7) (Slemmer *et al*., 2008). Activation of Ca^{2+}-dependent endonucleases can induce DNA cleavage that further causes cell death (Trump and Berezesky, 1995; McConkey

and Orrenius, 1996; Toescu, 1998). Calcium related mitochondrial damage is another pathway of cell destruction (Kristián and Siesjö, 1998). Calmodulin and Phospholipase A2 activation involves the production of excess free radicals post-injury, which causes cell damage (Slemmer *et al*., 2008). Other enzymes like caspases are also activated due to increased levels of Ca^{2+}. Induction of an apoptotic cascade takes place due to caspase activation (Slemmer *et al*., 2008).

The use of specific glutamate receptor blockers can inhibit intracellular calcium accumulation and reduce the calcium-induced oxidative stress. Also, antioxidants can be used to combat the oxidative stress condition and prevent further neurodegeneration.

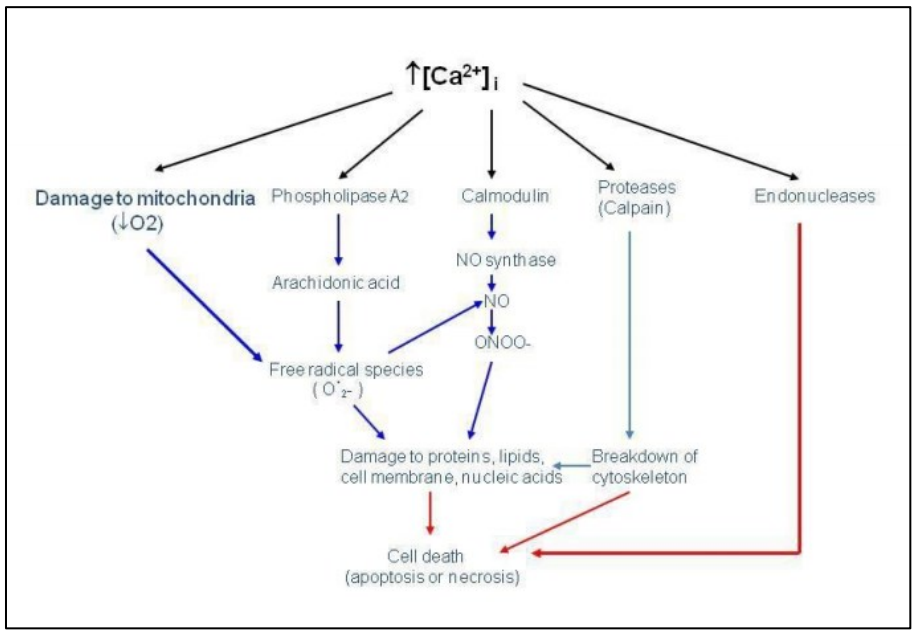

Figure 1.7: Calcium-induced oxidative stress and cell death (Modified from Weber., 2004).

1.9 Bioactive compounds in lingonberry

Bioactive compounds include phytochemicals found in foods that are capable of modulating metabolic processes, resulting in the promotion of better health by showing antioxidant activity, inhibition or induction of enzymes, inhibition of receptor activities, and

induction and inhibition of gene expression (Galanakis, in Nutraceutical and Functional Food Components, 2017). With the increase of the world population, there has been an increased need for healthy food availability. Food industries are now looking for new food sources every day that are not only tasty but also full of nutritious value. For the last few decades, berries have brought great attention to researchers because of their immense positive health effects. Now, berries are considered by many as one of the best available sources of bioactive compounds (Halvorsen *et al*., 2002, de Souza *et al*., 2014, Slatnar *et al*., 2012, Namiesnik *et al*., 2010). The chemical composition of each species of berry differs, and it can also vary depending on cultivar, growing location, environmental conditions, plant nutrition, ripeness stage and storage conditions. In general, all berries are rich in sugar and low in calories. They contain only a small amount of fat but are high in dietary fibre, organic acids, certain minerals in trace amounts, some vitamins and phytochemicals such as phenolic compounds (Kowalenko *et al*., 2005, Nile *et al.,* 2014, Skrovankova *et al*., 2015). Lingonberries are no exception. Similar to other *Vaccinium* species most of their beneficial health effects are due to the phenolic compounds present in fruits (Paredes-López *et al*., 2010). Several studies have identified a number of polyphenols such as polyphenolic acids, anthocyanins and procyanidins in lingonberries (Lätti *et al*., 2011, Ek *et al*., 2006, Leeet *et al.,* 2012, Hajazimi *et al.,* 2016, Tian *et al.,* 2017, Antolak *et al*., 2017). In addition to these nutrients, they also contain organic acids, vitamins (A, B1, B2, B3 and C), potassium, calcium, magnesium and phosphorous (Drózdz *et al.,* 2017).

1.9.1 Polyphenols and their classification

As previously discussed, dietary polyphenols have received tremendous attention from researchers and food industries due to some recent strong evidence that shows that polyphenols are capable of preventing neurodegenerative diseases, cardiovascular diseases and cancer

(Milner, 1994, Duthie and Brown, 1994). Also, polyphenols are well established as potent antioxidants (Williams *et al*., 2004, Dong, 2009). Chemically, polyphenols are a group of naturally occurring compounds with phenolic structures. Polyphenols are highly diverse and contain different subgroups of phenolic compounds (Figure 1.8). Foods that contain polyphenols include many vegetables, fruits, whole grains and beverages such as tea, coffee, wine and even chocolates (Tsao, 2010). Polyphenols are the largest and most widely distributed natural products in the plant kingdom. There are more than 8000 phenolic structures currently known and more than 4000 flavonoids have been characterized (Harborne *et al*., 2000, Bravo *et al*., 1998, Cheynier *et al*., 2005). Polyphenols can be classified according to their source of origin, biological function, and chemical structure (Tsao, 2010).

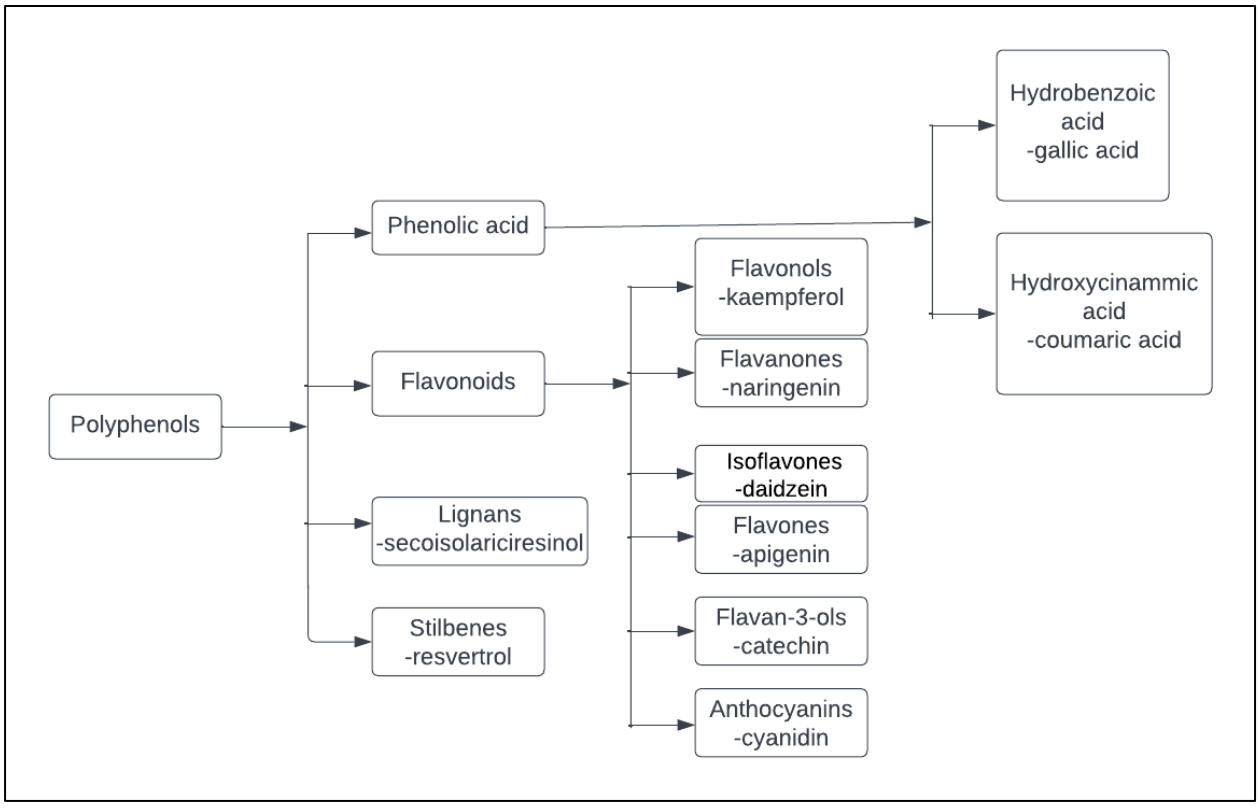

Figure 1.8: Classification of polyphenols and examples (modified from Hardman., 2014; drawn with Lucid chart)

1.9.1.1 Flavonoids

Flavonoids are naturally found compounds having polyphenolic groups in their structure and are mostly found in fruits, vegetables, grains, bark, roots and tea (Panche *et al*., 2016). Flavonoids contain two benzene rings, A & B where they are connected by a heterocycle pyrene ring C (Dias *et al.,* 2021). Flavonoids are subdivided based on the hydroxylation pattern in the C ring such as anthocyanins, flavan-3-ols, flavones, flavanones and flavonols. In most of the flavonoid structure ring B attaches at the C2 position of Ring C (Figure 1.9). Some flavonoids have their ring B attach at the C3 or C4 position, as example isoflavones and neoflavonoids respectively. Chalcones are also categorized as flavonoid though the heterocycle ring is absent (Tsao, 2010).

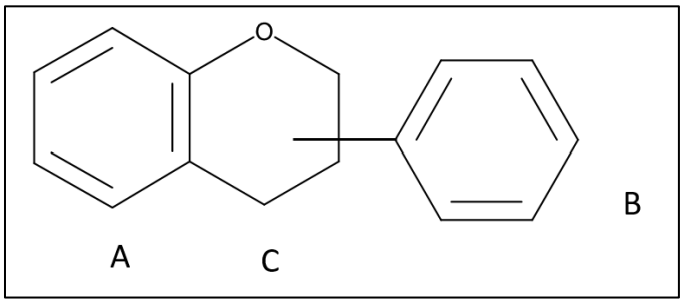

Figure 1.9: Basic backbone of flavonoid structures (drawn with Chem4word)

Flavonoids have effects both on plants and on human health. They play an important role in producing colours and aroma in flowers and contribute to pollination (Griesbach, 2005). A summary of their positive health effects on humans is represented in Figure 1.10.

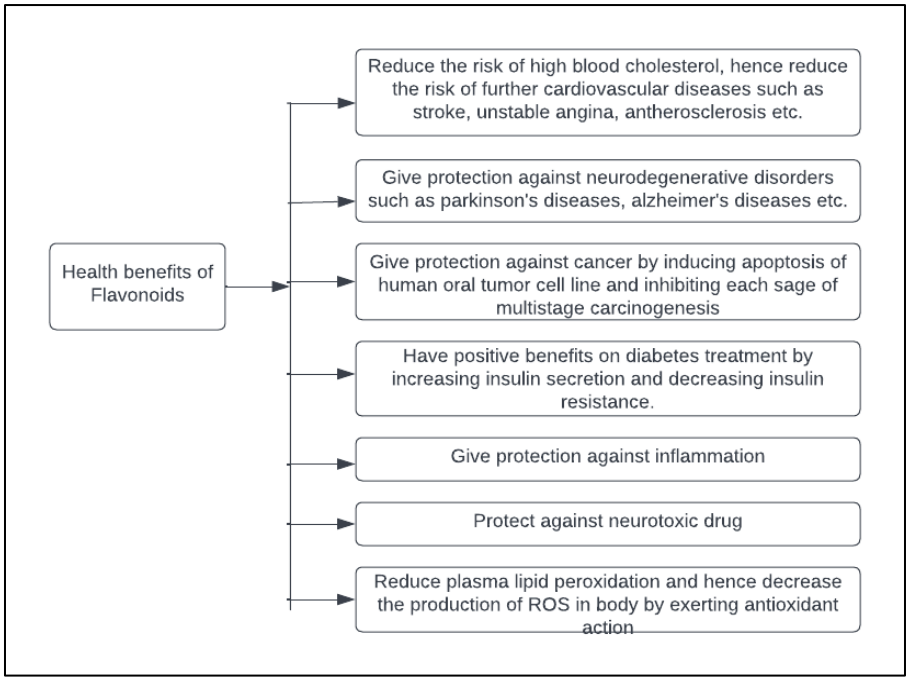

Figure 1.10: Some beneficial effects of flavonoids (modified from, Sharma & Thakur., 2018; drawn with Lucidchart).

1.9.1.2 Anthocyanidins

Anthocyanidins (Figure 1.11) are the main compounds responsible for colours in plants. The most common anthocyanidins are cyanidin, delphinidin, pelargonidin and there are a total of 31 anthocyanidins known (Anderson *et al*., 2006). Anthocyanidins are the aglycon part of anthocyanins which remain as glycosides in plants (Khoo *et al*., 2017). More than 500 anthocyanins are known, which are named depending on hydroxylation, methoxylation patterns between the rings and glycosylation with different sugar units (Tsao and McCallum., 2009 McCallum *et al*., 2007).

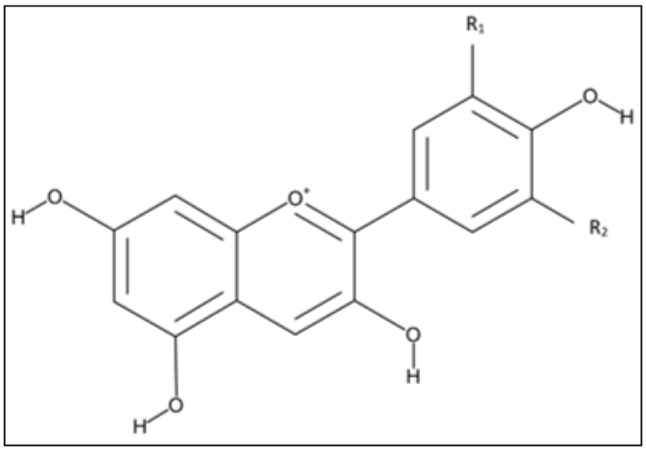

Figure 1.11: Basic structure of Anthocyanidins (drawn with Chem4word)

Table 1.1: A list of some anthocyanidins based on R_1 and R_2. (See Figure 1.11)

Anthocyanidins	R_1	R_2
Cyanidin	-OH	-H
Delphinidin	-OH	-H
Pergonidin	-H	-H
Malvidin	$-OCH_3$	$-OCH_3$
Peonodin	$-OCH_3$	-H
Petunidin	-OH	$-OCH_3$

Although anthocyanidins are not essential bioactive compounds and there are no deficiency incidences for human health, they help to maintain a healthy status throughout life. Anthocyanidins are also helpful to lower low-density cholesterol (Wallace and Giusti., 2015).

1.9.1.3 Tannins

Tannins are water soluble polyphenols naturally occurring in plants. Tannic acid is a specific tannin that usually contains 10 galloyl (3,4,5-trihydroxyphenyl) units surrounding a glucose center (Figure 1.12). Tannic acid contains no carboxyl groups but is weakly acidic because of the multiplicity of phenolic hydroxyls. The hydroxyls also cause it to be extremely soluble in water (Tanaka *et al.,* 2018). Tannins have antioxidant, anticarcinogenic and antimutagenic properties (Okuda and Ito, 2011), and can also prevent the growth of yeast, fungi

and bacteria. Tannins are used by food industries due to their antimicrobial properties (Singh and Kumar., 2019). Among other physiological effects of tannins are accelerating blood clotting, reducing blood pressure, decreasing serum lipid levels and modulating immune responses (Chung *et al*., 1998).

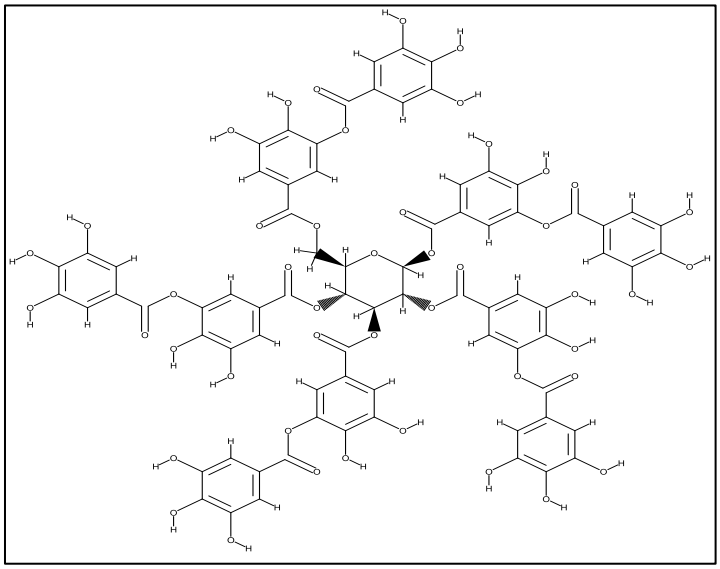

Figure 1.12: Structure of tannic acid (drawn with Chem4word)

1.9.2 Absorption, metabolism and bioavailability of polyphenols

Absorption and metabolism of polyphenols have been studied using radiolabelled atoms such as ^{14}C (Williamson., 2017). Most polyphenols in food are present as esters, glycosides or polymers, which cannot be absorbed in their native form and need to be hydrolyzed by enzymes or microbial flora. Polyphenols form conjugates via methylation, sulfonation and glucoronidation during absorption which facilitate biliary and urinary excretion by increasing hydrophilicity. During biliary excretion, polyphenols secrete into the duodenum and interact with bacterial enzymes such as β-glucuronidase and get reabsorbed, which ensures the prolonged presence of these compounds in the body (Manach *et al*., 2004). All flavonoids except flavonols remain in

their glycosylated forms and experiments have shown that glycosides are difficult to be absorbed in gastric areas, probably due to their resistance against hydrolysis in lower or acidic pH (Crespy *et al*., 2002, Piskula *et al*., 1999, Gee *et al*., 1998). Only aglycons and a few glucosides are absorbed from the intestine. Polyphenols which are linked with rhamnose reach the colon where they are hydrolyzed by rhamnosidase enzymes before absorption (Manach *et al*., 1995, Hollman *et al.,* 1997). Proanthocyanidin metabolism is very different than other classes of polyphenols as due to their high molecular weight it is difficult for them to be readily absorbed (Deprez *et al*., 2001). The suggested theory for proanthocyanidin metabolism is that they are hydrolyzed into a mixture of flavonols before absorption (Spencer *et al*., 2000). Because of their poor absorption most proanthocyanidins might exert local activities in the intestine such as protecting it from oxidizing agents, which induce inflammation and trigger cancer (Halliwell *et al*., 2000).

Various studies have shown that the bioavailability of polyphenols orally is not very high due to poor absorption, instability, excessive metabolism or intestinal microbial transformation (Cherubini *et al*., 1999). Despite their low bioavailability there is still evidence that they can show pharmacological activity in low plasma concentrations (Nifli *et al*., 2005). Many approaches have now been improvised to increase bioavailability of polyphenols like chemical derivatization and modified formulation (e.g., particle size and/or additives) (Abourashed *et al.,* 2013). Recent experiments have shown that metabolites from parent polyphenols are able to cross the blood brain barrier (BBB) endothelium at physiological concentrations and the endothelium converts metabolites into novel compounds, which exert different neurological activities in brain tissue (Figueira *et al.,* 2017).

Despite a lot of evidence on the bioavailability of polyphenols in the systemic circulation, there is not much evidence of their presence in the brain. To access the brain, they must need to

cross the BBB (Abbott *et al*., 2010). *In vitro* experiments have shown that the permeability of polyphenols greatly depends on their lipophilicity where less polar molecules have more possibility to cross the BBB than polar compounds (Youdim *et al.,* 2003). Bioavailability in the brain also depends on the efflux transporters of the brain and the compound's stereochemistry (Youdim *et al*., 2004, Faria *et al*., 2011). Animal model experiments also showed that the presence of polyphenols in brain tissue is independent of their route of administration (Peng *et al.,* 1998). Although there is strong evidence of the BBB's uptake and distribution of dietary polyphenols in the brain, the amount reaching different parts is still uncertain. A recent mathematical correction model has shown that polyphenols localization in the brain after distribution is below one nmol/g of tissue (Schaffer and Halliwell., 2012). Several other studies also demonstrated the presence of polyphenols in different regions of rat and pig brain (Talavéra *et al.,* 2005, Passamonti *et al.,* 2005, Kalt *et al.,* 2008 and Milbury *et al*., 2010). All of the studies demonstrating the presence of dietary polyphenols in brain tissue raise the hope of researchers in that they may be potential candidates for direct neuroprotective effects and neuromodulatory actions.

1.10 Hypothesis

Considering the immense evidence from *in vitro* and *in vivo* studies that lingonberries are capable of exerting high free radical scavenging properties, anti-inflammatory, anti-microbial, antimutagenic and anti-cancer activities, one can assume that there is the presence of bioactive compounds, which are responsible for these beneficial effects. Also, studies show that lingonberries can delay the ageing process and onset of neurodegenerative disorders, which are very characteristic properties of polyphenols. Considering all of this information, I hypothesized

that wild lingonberries from Fogo Island contain polyphenols such as anthocyanins, tannins and flavonoids in an almost equal or higher concentration than other types of berries.

1.11 Objectives

The objectives of this research are:

- To perform a comparative analysis of different classes of polyphenols present in various berries including blueberries, lingonberries, crowberries and bunchberries collected on Fogo Island, NL.

- To determine the free radical scavenging capacities of the fruits and leaves of different berries.

- To perform a comparative analysis of total phenolic, tannin and anthocyanin content and determine antioxidant capacities in lingonberries collected from various locations on Fogo Island, NL.

-To establish an analytical method for qualitative and quantitative analysis of lingonberry samples.

- To characterize the compounds found in lingonberry samples by qualitative and quantitative analysis.

Chapter 2: Materials and Methods

2.1 Plant extraction procedure

2.1.1: Collection and storage

Four kinds of berries, both leaves and fruits, were collected from six different locations (see Table 2.1) on Fogo Island in two consecutive years (2017 and 2018) in the fall season. These species included blueberries, lingonberries, crowberries and bunchberries. All of the samples collected were stored at -20°C within the first hour of collection. Samples were then stored in a -80°C freezer to prevent degradation of bioactive compounds as it has been found that phenolic content remains or increase in cold storage due to phenolic accumulation (Connor *et al*., 2002).

Table 2.1 Geographical information on collection sites of berries from Fogo Island

Location	Latitude	Longitude
1.Island Harbour	49.6220˚N	54.3086˚W
2. Lion's Den	49.7201 ˚N	54.2616 ˚W
3. Joe Batt's Point	49.7385 ˚N	54.1554 ˚W
4. Marconi Site	49.7191 ˚N	54.2622 ˚W
5. Seldom	49.3700 ˚N	54.1116 ˚W
6. Tilting	49.4200 ˚N	54.0400 ˚W

2.1.2 Fruit and leaf extraction

Stored fruits and leaves were removed from the freezer and thawed at room temperature. Debris, such as stems and dirt were removed, as well as extensively damaged berries. Extraction solvent was prepared consisting of 80% (v/v) acetone, 0.2% formic acid, and 19.8% distilled water (dH_2O). The samples were placed in a mortar and mashed. Weight was measured, and then extraction solvent was added to the ground samples to a concentration of 1:2 (v/w). The mixture was then vortexed for 20 seconds, placed in a cooler with ice and shaken for 30 minutes. The mixture was then centrifuged in a centrifugal machine at 4°C for 20 minutes at 10000 g. The supernatant was then drained into a separate container, and the whole procedure was repeated using the remaining residue. The final concentration was 250 mg/ml solvent of fresh weight, which is the weight of the specimen including its water content. The procedure was consistent with previous studies in the lab (Vyas *et al*., 2013).

2.2 Equipment

To prepare samples, a centrifugal machine, Model: Allegra 64R was used to collect the supernatant. For biochemical assays, a Libra S32 PC UV/Visible spectrometer was used to record absorbance. To prepare samples for HPLC, the prepared samples were freeze-dried using a Labconco freezone 12 freeze dryer. The Agilent 1260 HPLC- 1260 DAD- 6230B MSD TOF analyzer was used for chemical analysis of lingonberry fruits and leaves.

2.3 Chemicals and reagents

Reagents that were used in different experiments are acetone, formic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Folin–Ciocalteau reagent, catechin, vanillin, aluminium chloride, ascorbic acid, potassium chloride, sodium nitrite, methanol, sodium acetate, sodium

hydroxide and cyanidin 3- galactoside. Chemicals were purchased from Sigma-Aldrich, Canada Ltd and Fisher scientific Ltd.

2.4 Biochemical assays

All stock samples of fruit and leaf extracts were analyzed within one week of extraction, and all experiments were repeated three times for each sample. All the samples were stored in the -80°C freezer before and after each experiment. Further dilution was necessary for the stock extracts at 1:10 (stock:solvent) for the fruit and 1:100 for the leaves, to a final concentration of 25 mg/ml of fruit and 2.5 mg/ml of leaf fresh weight. dH_2O was used for the blank and to rinse out the quartz cubes between samples. All assays followed the same methods described by Vyas *et al.* (2013) & Debnath-Canning *et al.* (2020).

2.4.1 Total antioxidant capacity

Total antioxidant capacity was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. DPPH is a stable free radical which is violet in color, but when it is neutralized, a colorimetric reaction occurs that makes the solution lighter in color. This method allows for the quantification of free radical scavenging ability of the sample, as antioxidants are able to neutralize the radical DPPH. The method was adapted from Vyas *et al.* (2013), and Brand-Williams *et al.* (1995), where 100 µl of berry extract was added to 1.9 ml of 0.04 mM stock DPPH. The solution was then vortexed for 20 seconds, protected from light using aluminium foil for 20 minutes and measured with a UV/Visible spectrometer at 517 nm. Total antioxidant capacity is expressed as '%' of inhibition of DPPH consumption, and the results are expressed as 'Gallic Acid Equivalents' (GAE). Gallic acid was used as standard due to its availability and easy solubility in water.

2.4.2 Total phenolic content (TPC)

In order to determine the TPC of the berry fruit and leaf extracts, Folin-Ciocalteu reagent was used. Folin-Ciocalteu is a redox reagent that is blue in color, the higher the phenolic content the darker the blue. Blueberry extract of 0.5 mL was added to 0.5 ml of Folin-Ciocalteu reagent. Then, 1 mL of sodium carbonate (Na_2CO_3; 25 g of Na_2CO_3 into 250 ml dH_2O) and 8 ml of dH_2O was added to the solution and vortexed for 30 seconds. The solution was protected from light using aluminium foil for 35 minutes, and then centrifuged at room temperature for 10 minutes at 1000 g. Absorbance was measured at 725 nm using a UV/Visible spectrophotometer. TPC was then determined using a gallic acid standard curve, and the results are expressed as milligrams of GAE per gram of fresh weight. The method for this assay was adapted from Chandrasekara and Shahidi (2011).

2.4.3 Total flavonoid content (TFC)

To determine the TFC of the samples, 1 mL of extract was added to 4 ml of dH_2O, and 0.3 ml of 5% sodium nitrite ($NaNO_2$) and left to sit for 5 minutes. Then, 0.3 ml of 10% aluminum chloride ($AlCl_3$) was added, and one minute later 2 ml of sodium hydroxide (NaOH) and 24 ml of dH_2O were mixed in. The solution was protected from light for 15 minutes, and then centrifuged at room temperature for 4 minutes at 4000 g. The absorbance was measured at 510 nm with a UV/Visible spectrophotometer. The method was adapted from Zhishen *et al.* (1999), and the results are expressed as milligrams of catechin equivalents (CE) per gram of sample.

2.4.4 Total tannin content (TTC)

TTC was determined using vanillin-hydrogen chloride reagent (0.5g of vanillin added to 96 ml methanol [MeOH], plus 4 ml of HCl). For this assay, 1 ml of blueberry extract was added

to 5 ml of 9.5% vanillin-HCl reagent which produced a bright red color. The solution was then incubated and protected from light for 20 minutes using aluminium foil and the absorbance was measured at 500 nm using a UV/Visible spectrophotometer. The results were expressed as milligrams of CE per gram of sample. The method for TTC was adapted from Chandrasekara and Shahidi (2011).

2.4.5 Total anthocyanin content (TAC)

To determine TAC two buffer solution were used which were potassium chloride (KCl) and sodium acetate ($NaCO_2CH_3$) with hydrochloric acid. To make potassium chloride buffer of pH 1 and 0.025 M was made by adding 1.86 g of potassium chloride and then hydrochloric acid in water. Hydrochloric acid wad added to adjust the pH to 1. Sodium acetate buffer of pH 4.5 and 0.4 M was made by adding 54.43 g of Sodium acetate in water and then adjust the pH to 4.5 by adding acetic acid. For experiment, one ml of berry extract was added to 4 ml of $KCl/NaCO_2CH_3$ buffer for a total volume of 5 ml and placed in the dark using aluminium foil for 15 minutes. The absorbance was measured at both 510 nm and 700 nm using a spectrometer. The corrected absorbance result was calculated as ($[A510 - A700]_{pH=1.0} - [A510 - A700]_{pH=4.5}$). The method was adapted from Foley and Debnath (2007), and the results are expressed as GAE.

2.4.6 Standard curve for biochemical assays

A standard curve was prepared for calculation of each biochemical assay using absorbance given by standard solution over different concentration of 50,100, 150, 200 and 250 µg/ml. A linear equation was derived (y=mx+c) from curve with $R^2 \geq 0.99$.

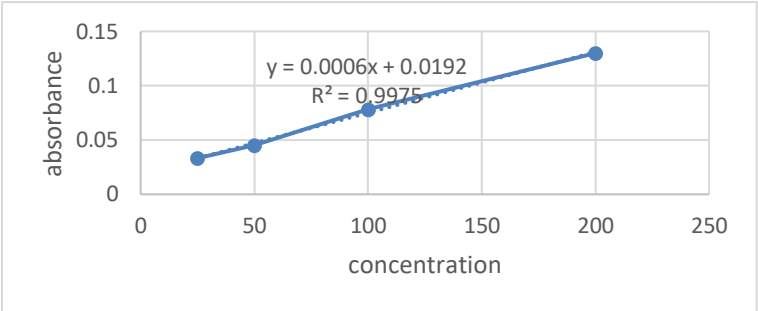

Figure 2.1: Representative graph of a standard curve.

2.5 HPLC and MS analysis of lingonberries

To determine which polyphenols were present in the leaf and fruit extracts, HPLC-MS analysis was conducted for some lingonberry samples. From previous research conducted in our lab it was shown that wild blueberry has a significant number of polyphenols and cell culture studies of wild blueberry samples in our lab showed that they can potentially decrease inflammation and cell death (Debnath-Canning et al., 2020). Research conducted by another lab member showed that lingonberry extracts also have inhibited cell death in an *in vitr*o model of cell injury (Hossain et al., 2016), which might be the result of the presence of a number of anthocyanins and flavonoids detected in lingonberry samples. In this project, we chose to focus on anthocyanins in lingonberries. To simplify the analysis here we narrowed down the focus to compounds available in lingonberries collected on Fogo Island. In order to quantify the amount of certain anthocyanins detectable in the leaves and fruits, cyanidin 3-galactoside was used as a standard.

2.5.1 Sample preparation for HPLC

The preparation of extracts was performed using methods outlined by Cho *et al.* (2004) with modifications. Lingonberry samples were homogenized into a paste using a mortar and

pestle. Leaf samples were ground into a fine powder using liquid nitrogen. Homogenate was placed into a lyophilizer and freeze-dried overnight until dry. Twenty ml of extraction solution (methanol/water/formic acid 60:37:3 v/v/v) was mixed with 5 g of fruit/leaf powder. This mixture was then sonicated for 30 minutes in a water-bath sonicator, and then centrifuged at 2600 g for 30 minutes at 4°C. A 4 ml aliquot of the supernatant was collected and placed in a rotary evaporator at 45 °C until at least 95% of extraction solvent was removed. The sample was then re-suspended in 1 ml of 3% formic acid in water solution and filtered through a 0.45 µm pore filter.

2.5.2 Standard curve for HPLC

Two grams of anthocyanin reference standard (cyanidin-3-galactoside) was dissolved in 10 ml of 3% formic acid to make a final concentration of 200 µg/ml. Serial dilutions were conducted to obtain concentrations of 150 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml and 12.5 µg/ml of the reference standard stock solutions. Each standard solution was injected separately under the same HPLC conditions in order to create calibration curves for the reference compounds. The aim was to generate a linear calibration curve with $R^2 \geq 0.99$.

2.5.3 HPLC parameters

A mass spectrometer was used with HPLC, which uses time of flight (TOF) of ions to determine their mass, named the Agilent 1260 Infinity LC-6230 TOF LC/MS system. Fruit and leaf extracts from the location, which showed the highest levels of polyphenols in the biochemical analysis, were injected (about 15 µl) into the HPLC system. Separation was conducted using a 3.9 mm x 150 mm C18 (5 µm) column. Prior to each injection, the system was equilibrated for 20 minutes. A Diode Array Detector (DAD) was used to detect flavonols at 360 nm and anthocyanins at 520 nm. The liquid chromatography flow rate was at 0.8 ml/min and the

temperature was at 25 °C. The mobile phase consisted of a linear gradient of 1% formic acid (A) and methanol (B) from 12% methanol to 55% methanol for 25 min at a rate of one ml/min. The ratio of solvent A and B was fixed by trial and error to see which ratio gave the best results.

2.5.4 MS parameters

The parameters for MS were similar as developed by Cho *et al.* (2004), where both positive and negative electrospray ionization (ESI) were utilized. The capillary voltage was 4.0 kV, nebulizing pressure of 30.0 psi, temperature of 300 °C, drying gas flow rate of 9.0 ml/min. The performance was at 1.0 second/cycle with a scanner mass range of 10-1000 m/z (mass/charge). For identification of tentatively found compounds, HPLC chromatographs and their MS values were compared to values obtained in the literature.

2.6 Statistical analysis

Each experiment included three biological samples that were tested in triplicate, consistent with previous studies in the lab (Vyas *et al*., 2013; Debnath-Canning *et al*., 2020). All of the results are expressed as mean ± standard error (SE) of all three replicates. Using two-sample assuming unequal variance t-test (Microsoft Excel), statistically significant differences were determined between fruits and leaves. One-way ANOVA and Duncan's Multiple Range Variance Test (SPSS 39, IBM Inc.) were used to determine differences between different locations.

Chapter 3: Results

3.1 Biochemical Assays

Four types of berries, blueberries, lingonberries, crowberries and bunchberries were collected from six locations on Fogo Island, Newfoundland. Samples were collected from different regions of the island to observe the variation in quantities of polyphenols present in berries of the same species due to the slight variations in climate, sun exposure, soil and environment. Also, samples were collected for two consecutive years, 2017 and 2018, during the Fall season in order to compare differences of one year. In 2017 blueberries, lingonberries, crowberries and bunchberries were collected from six locations on Fogo Island which are Seldom, Island Harbour, Tilting, Marconi Site, Joe Batt's pt. Trail and Lion's Den. In 2018 samples were collected from four location which are Seldom, Island Harbour, Tilting and Simm's Beach due to unavailability of samples from other locations. Samples have been collected in triplicate from each location to make more accurate statistical comparison. Fruits and leaves of each berry species have been assessed with each biochemical assay individually. For each biochemical assay, all of the samples of the four mentioned types of berries have been compared for the two-year periods of collection. A comparative study has been conducted among blueberries, lingonberries, crowberries and bunchberries in terms of the presence of nutraceuticals and antioxidant capacity. For the graphical representation to compare data from each year here we have used mean value ± Standard Error as samples were collected from different regions for each year. Data from each assay were compared statistically. We only focused here on lingonberries in more detail due to its previously mentioned potential health benefits and also because our lab already has extensive data on chemical and biological activities

of blueberries (Vyas *et al*., 2013, Debnath-Canning *et al.*, 2020). Lingonberries from different regions have been assessed and graphed for each biochemical test and each of the two years. It has been shown that the number of polyphenols and antioxidants activities vary depending on location, type of sample (fruits or leaves) and year. Data have been analyzed statistically to determine the significant differences using t-test assuming unequal variances ($P< 0.05$). The significant difference between samples of different locations was calculated by One-way ANOVA, followed by Duncan's Multiple Range Test. All of the results have been expressed as mean ±SE where experiments were performed for three biological replicates. Within the same column the same letter with same number (ex. A1 and A1, or, B1 and B1) has been used to indicate no statistical significance but same letter with different number (ex. A1 and A2) indicates statistical significance at $P < 0.05$.

3.1.1 TPC

In the TPC assay statistical analysis showed leaves have higher phenolic content than fruits. Also, samples of leaves collected in 2018 have a higher value than from 2017. Blueberry fruits and leaves from 2018 showed statistically higher phenolic content than other berries. Figure 3.1 shows the comparison among blueberry, lingonberry, crowberry and bunchberry fruits and leaves from 2017 and 2018 in terms of TPC. Blueberry and lingonberry fruits have no significant differences between the two years. However, all the leaves samples and crowberry fruits gave significantly different results based on years.

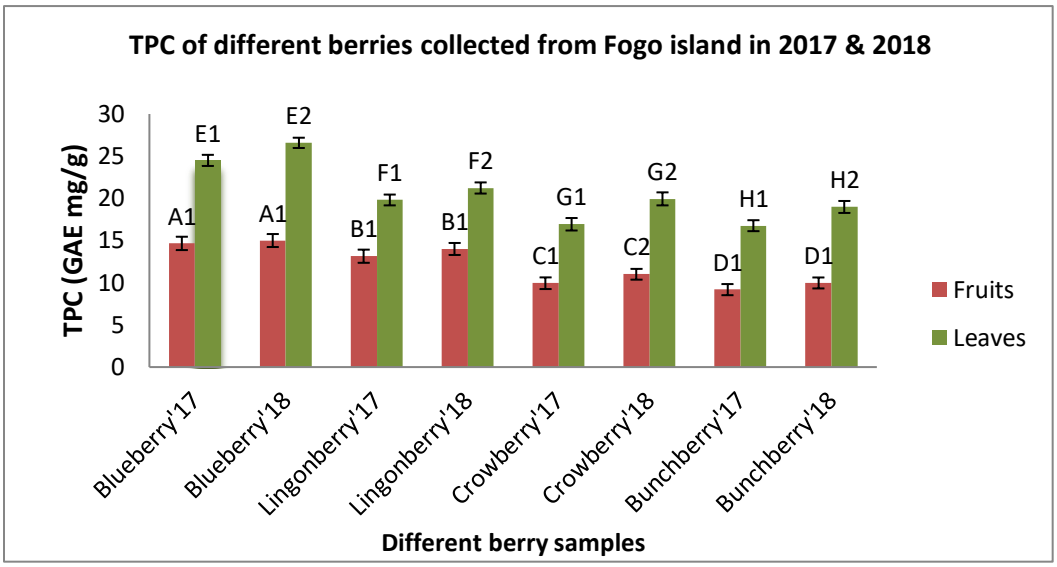

Figure 3.1: TPC in fruits and leaves of blueberry, lingonberry, crowberry and bunchberry collected in 2017 and 2018.

The data are expressed as the mean ± SE. Letters, A, B, C, D, E, F, G and H have been used to express statistical data of blueberry fruits, lingonberry fruits, crowberry fruits, bunchberry fruits, blueberry leaves, lingonberry leaves, crowberry leaves and bunchberry leaves accordingly. The same letter with the same number indicates no statistical significance of two samples from two different years. The same letter but different number indicates statistical significance where $p < 0.05$. GAE: Gallic Acid Equivilant.

In Figures 3.2 and 3.3, we statistically analyzed TPC in various lingonberry fruits and leaves from 2017 and 2018. In the first graph (Figure 3.2), fruits from Island Harbour have the highest content and differences with others are not statistically significant except for Tilting and Lion's Den. Leaves collected from Tilting show the highest amount of phenolic content but is also similar with data found from other places. In the later graph (Figure 3.3), leaf samples collected from Island Harbour have the highest value and the difference between this data and data from

other places are statistically significant. Fruits from Simm's Beach have shown the highest phenolic content and the difference with other samples are statistically significant.

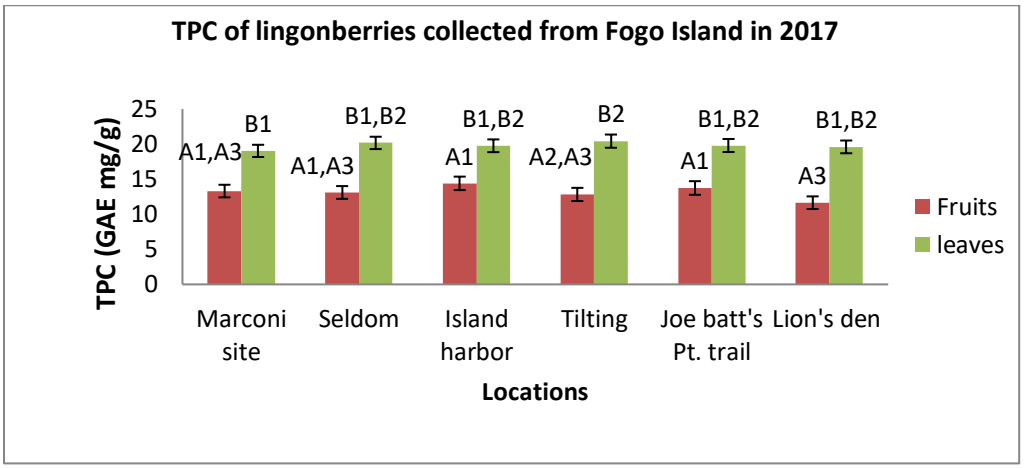

Figure 3.2: TPC in fruits and leaves from lingonberry samples collected from six different locations in 2017.

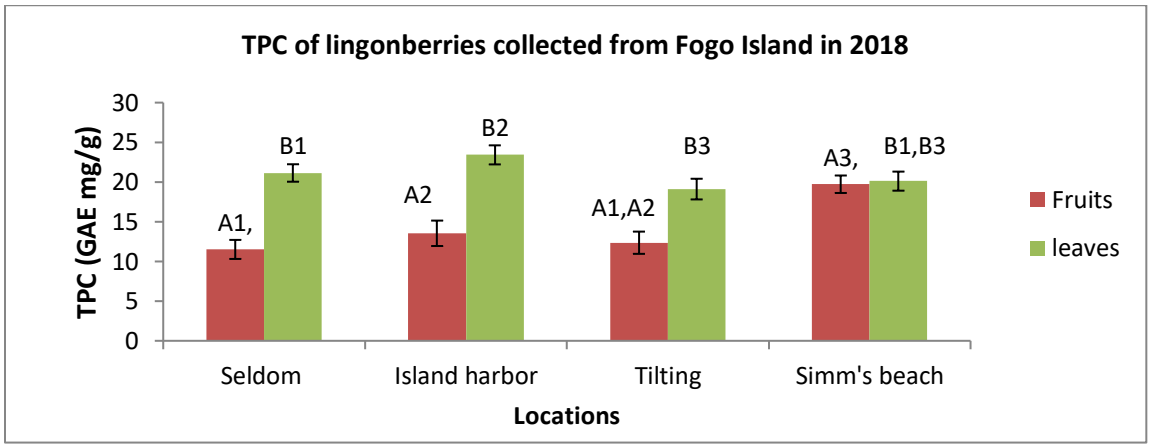

Figure 3.3: TPC in fruits and leaves from lingonberry samples collected from four different locations in 2018.

3.1.2 TFC

In the TFC assay, statistical analysis showed, overall leaves have higher values than fruit samples (Figure 3.4). Samples collected in 2018 have a higher number of flavonoids than samples

collected in 2017, but the result is not significant. Blueberry leaves have higher flavonoid content compared to other species and lingonberry fruits have the highest value among fruit samples. In figure 3.4 four types of berries have been compared for two years where all leaves samples are statistically not significant except crowberries. Also, all fruits samples of each berry from two years are not statistically significant. In Figures 3.5 and 3.6 lingonberry samples from different locations have been compared. Fruit samples collected in 2017 from different locations almost have similar flavonoid content and differences among them are not statistically significant. Like 2018, fruit samples collected in 2017 also are not statistically significant, although some leaf samples are significantly different.

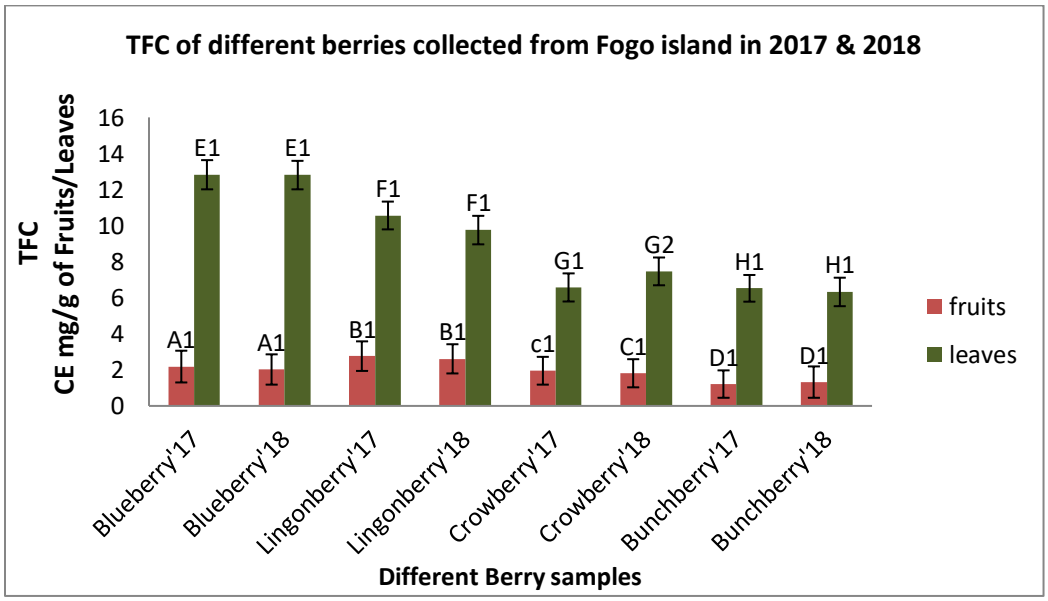

Figure 3.4: TFC in fruits and leaves from blueberry, lingonberry, crowberry and bunchberry samples collected in 2017 and 2018.

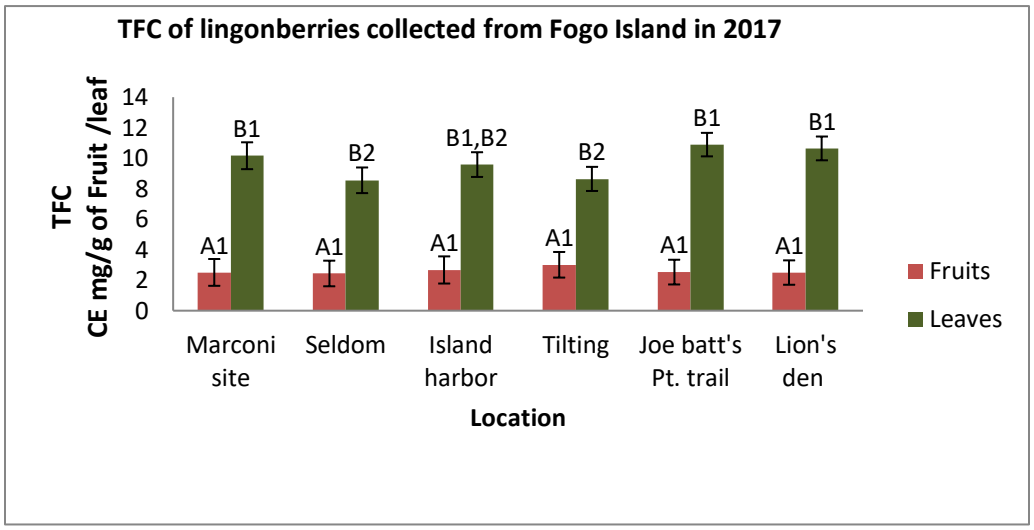

Figure 3.5: TFC in fruits and leaves from lingonberry samples collected from six locations in 2017.

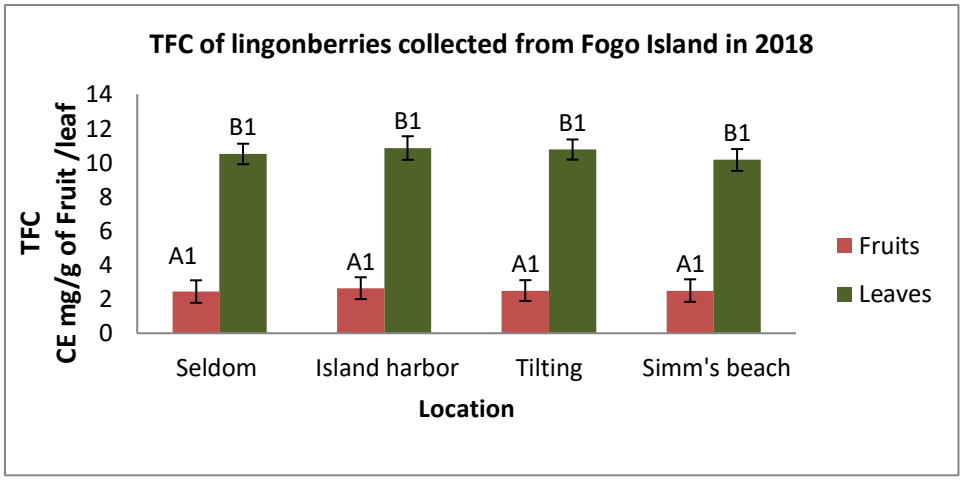

Figure 3.6: TFC in fruits and leaves of lingonberry extracts collected in four different locations in 2018.

3.1.3 TAC

Unlike the TPC and TFC assays, fruits had a higher anthocyanin content statistically than leaves in the total anthocyanin assay. Figures 3.7 shows total anthocyanin content of blueberry,

lingonberry, crowberry and bunchberry fruits and leaves. Crowberry leaves and fruits have a higher anthocyanin value than blueberry, lingonberry and bunchberry. Anthocyanin content value of fruit samples of all berries from each year are statistically significant, whereas the leaf sample from both years are not statistically significant. Figure 3.8 and 3.9 shows the comparison of anthocyanin content present in lingonberry fruits and leaves extract collected from different locations. Differences between data of fruit samples from 2018 have no statistical significance. However, differences between leaf samples collected from Seldom and other places are statistically significant.

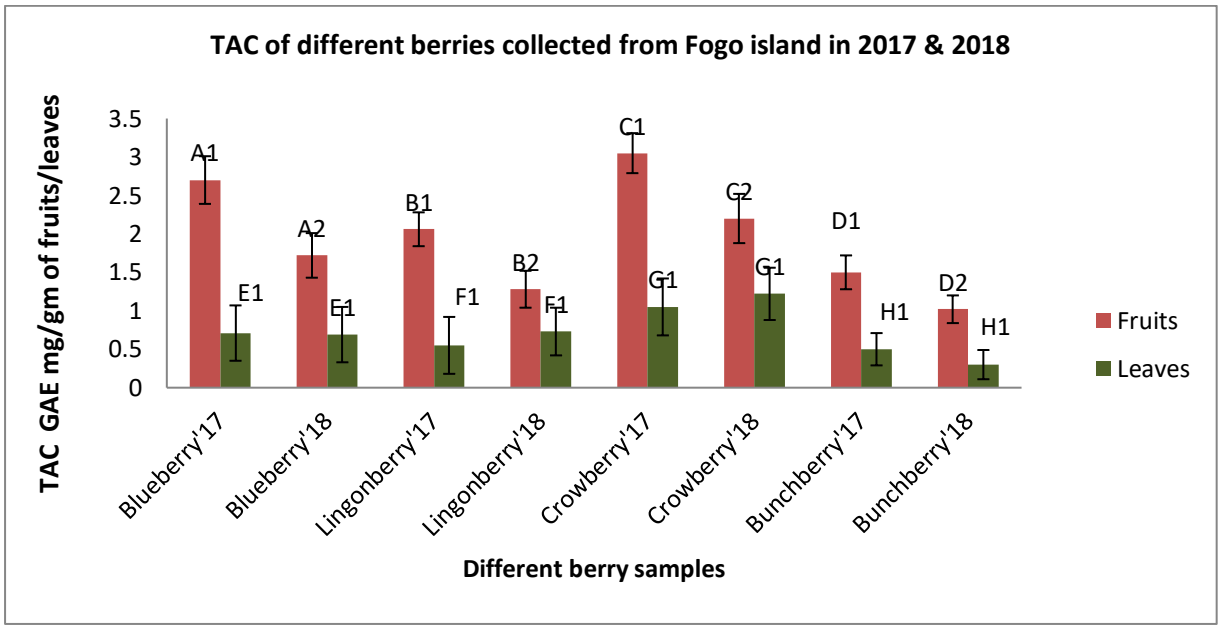

Figure 3.7: TAC in fruits and leaves of blueberry, lingonberry, crowberry and bunchberry from 2017 and 2018.

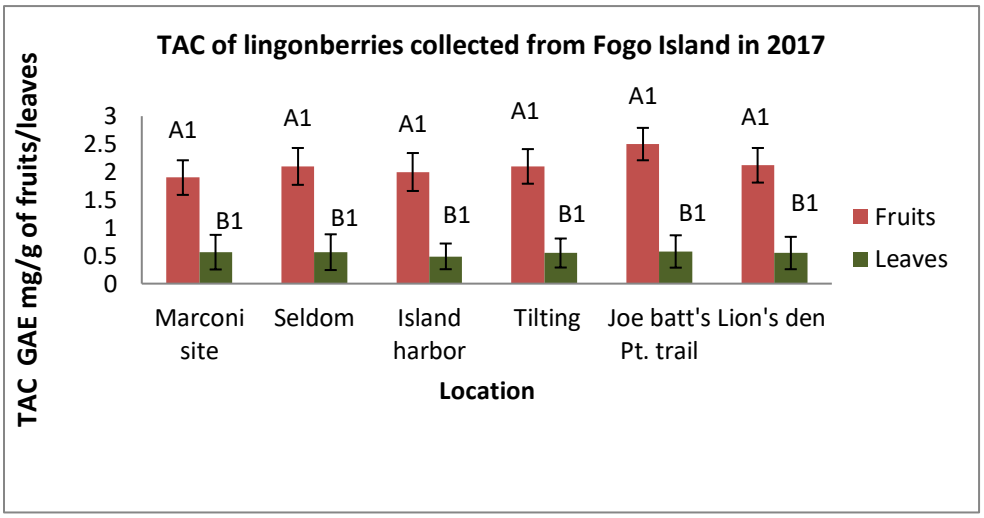

Figure 3.8: TAC in fruits and leaves of lingonberry extracts collected from six different locations in 2017.

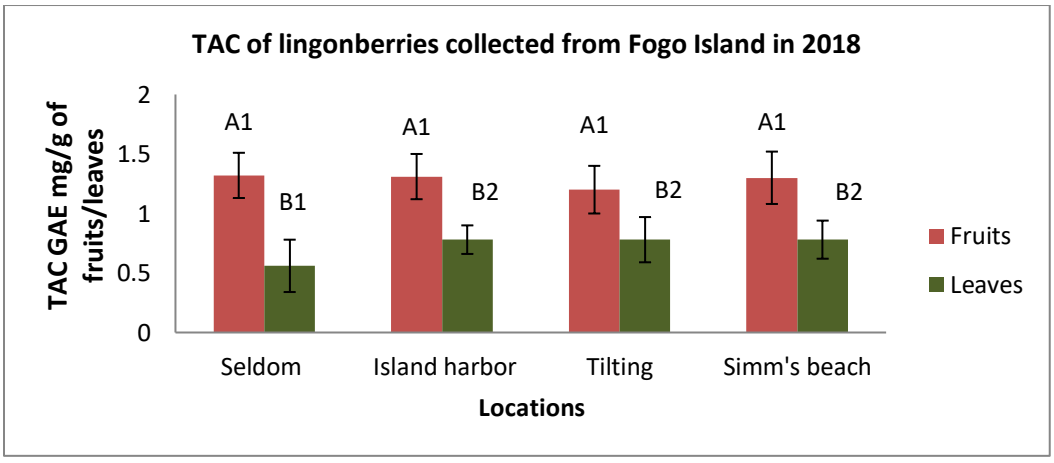

Figure 3.9: TAC in fruits and leaves of lingonberry extracts collected from four different locations in 2018.

3.1.4 TTC

As with the total anthocyanin assay, fruits have shown higher tannin content than leaves. From figure 3.10 statistics showed that crowberry fruits from 2017 have the highest tannin content among all fruits sample and blueberry leaf sample from 2017 has the highest among all leaf

samples. The difference between crowberry fruit samples of two years is statistically significant. Differences between blueberry and crowberry leaf samples of two years are also statistically significant. Figures 3.11 and 3.12 show that lingonberry fruits and leaves collected from Tilting have higher values in both years than other locations. It has also shown that the differences between samples from various locations are statistically significant for both years.

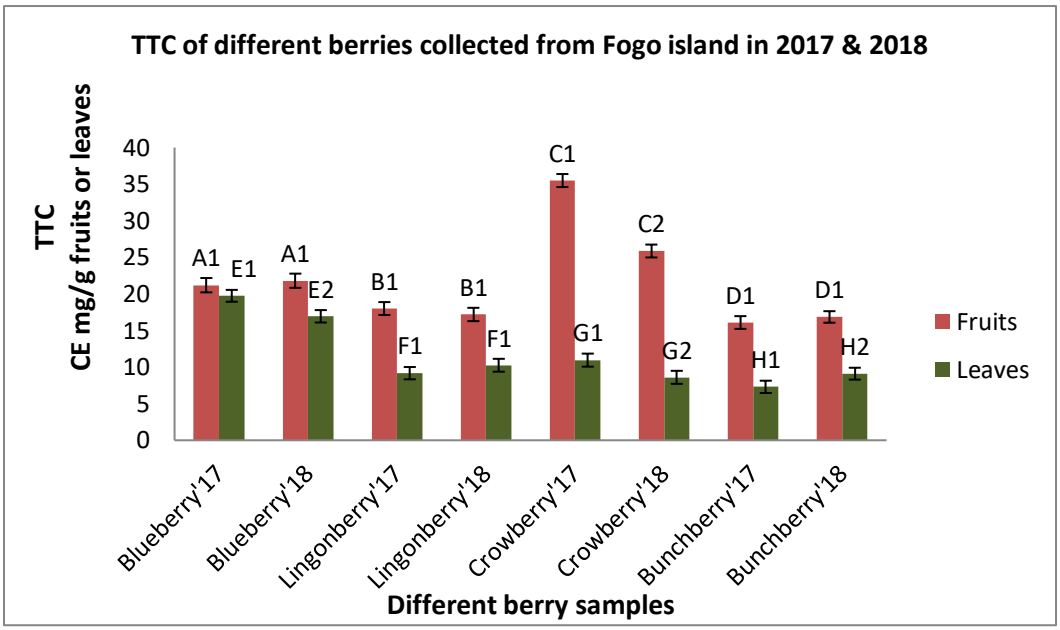

Figure 3.10: TTC in fruits and leaves of blueberry, lingonberry, crowberry and bunchberry from 2017 and 2018.

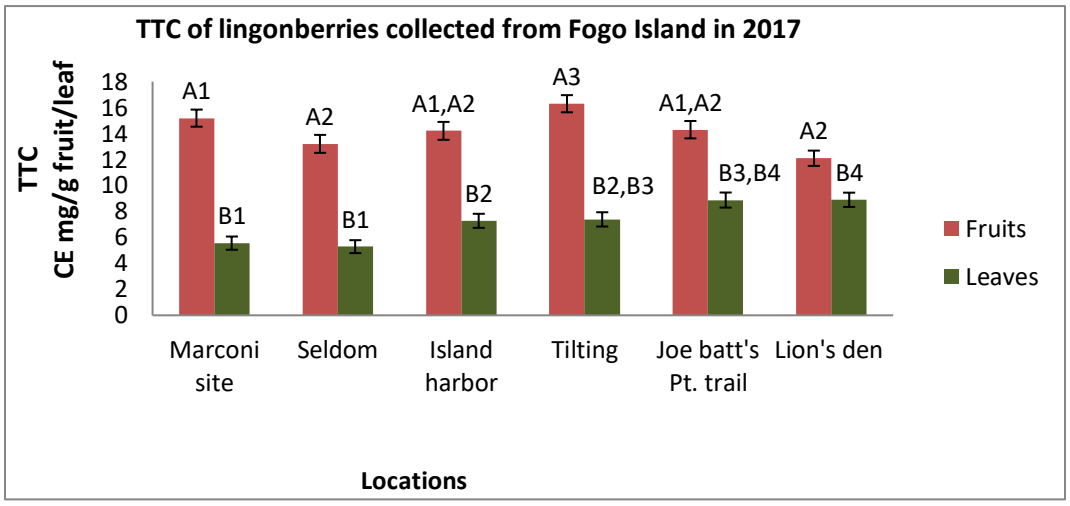

Figure 3.11: TTC in fruits and leaves of lingonberry samples collected from six different locations in 2017.

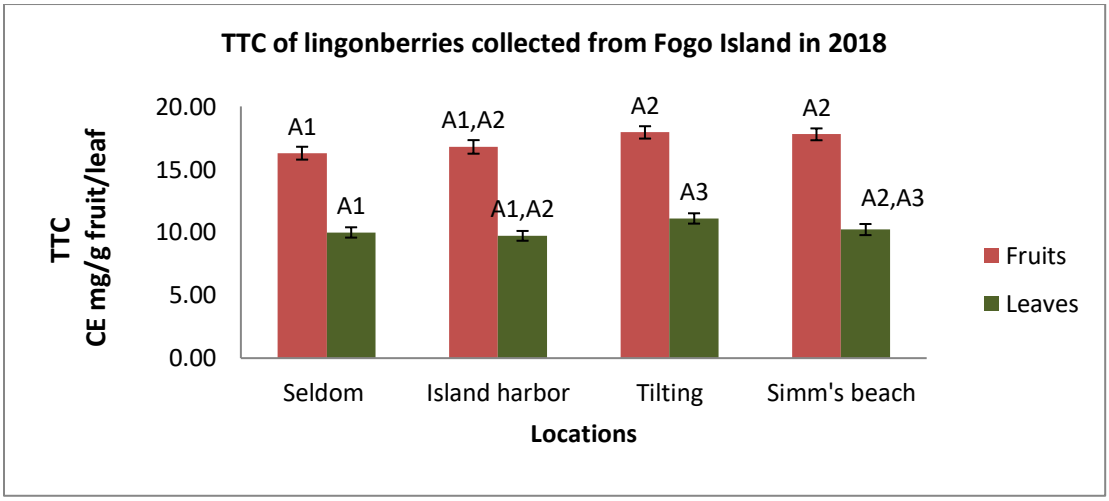

Figure 3.12: TTC in fruits and leaves of lingonberry samples collected from four different locations in 2018.

3.1.5 Free radical scavenging activity

Antioxidant activity has been measured in berry samples in terms of Diphenyl picrylhydrazyl (DPPH) free radical scavenging capacity. Figure 3.10 compares the free radical scavenging activities of all four types of berry samples from two years where both leaf and fruit samples of

2018 of blueberry indicated high antioxidant capacity. According to statistics, lingonberry has a higher antioxidant capacity than crowberry or bunchberry. Differences in samples from both years are not statistically significant except for lingonberry fruit samples. Overall, samples of leaves have higher free radical scavenging abilities than fruits. Figures 3.11 and 3.12 show the statistical analysis of the antioxidant capacities of lingonberry samples from both years collected from various locations. Extracts from samples collected from Simm's Beach have the highest value among all locations in 2018. Differences among all data from 2017 are not statistically significant.

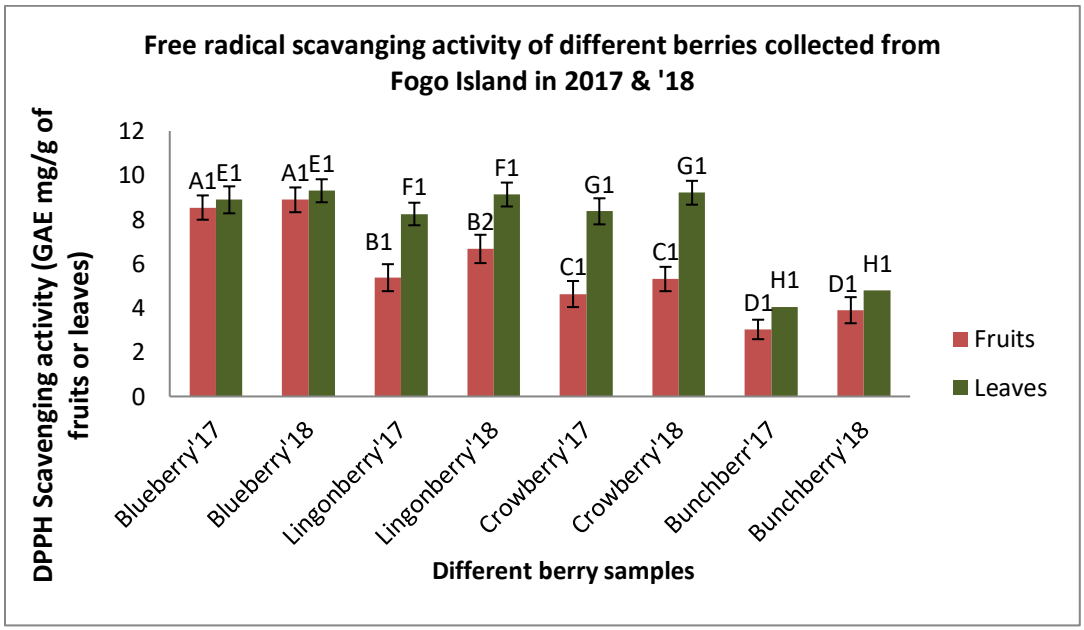

Figure 3.13: Diphenyl picrylhydrazyl (DPPH) free radical scavenging activities of fruits and leaves of blueberry, lingonberry, crowberry and bunchberry from 2017 and 2018.

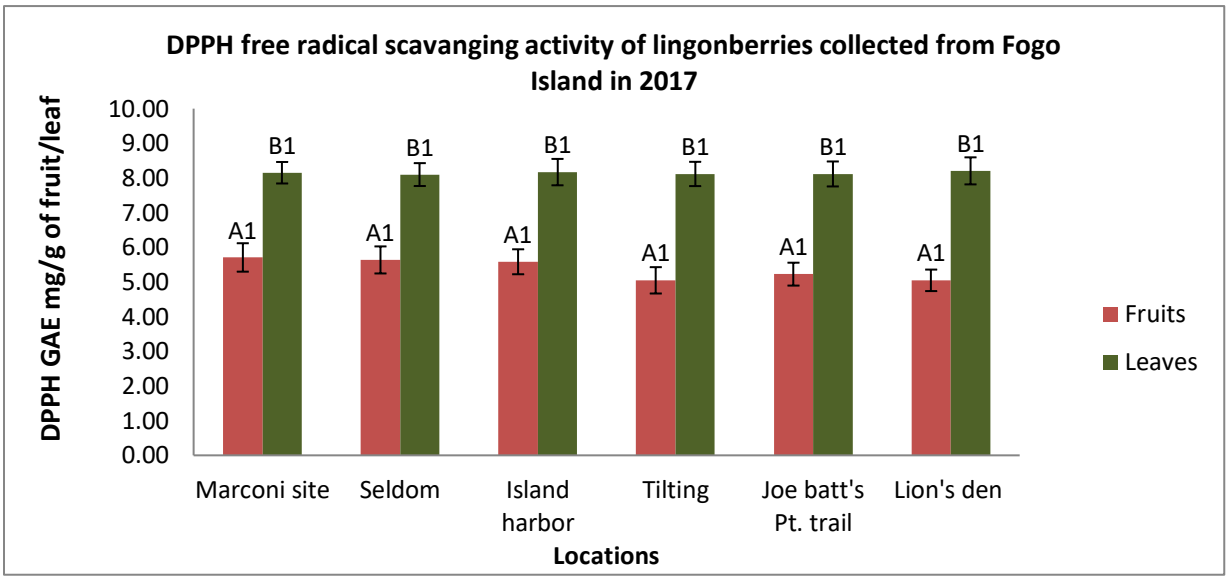

Figure 3.14: DPPH free radical scavenging activities of fruits and leaves of lingonberry samples collected from six different locations in 2017.

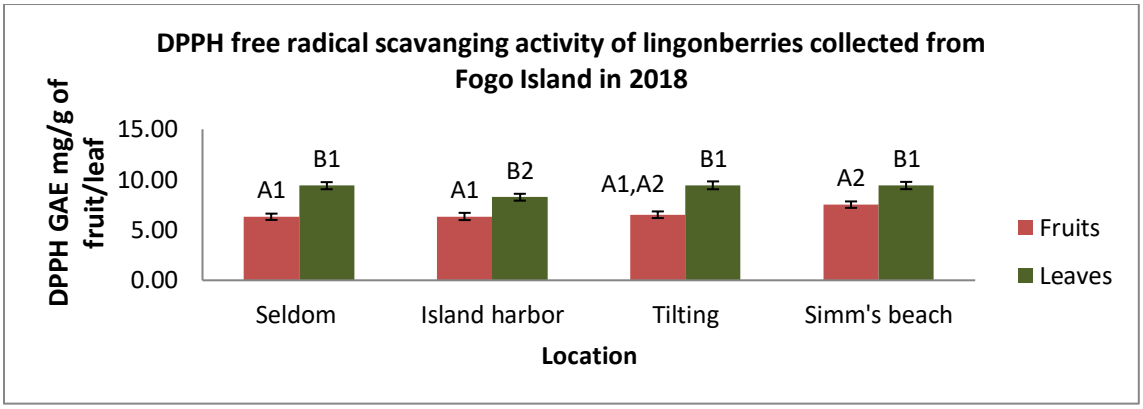

Figure 3.15: DPPH free radical scavenging activities of fruits and leaves of lingonberry extracts collected from four different locations in 2018.

3.2 Identification of the compounds in lingonberry fruit and leaf extracts and quantification of anthocyanin in extracts using HPLC and MS analysis

In this study more detailed chemical analysis of lingonberry extracts was carried out along with biochemical assays. We chose to further analyze the sample with high free radical

scavenging capacity, which were the samples from Simm's Beach in 2018 and from Lion's Den in 2017 respectively.

From previous studies it is well known that berries are rich in flavonoids. The flavonoids found in berries are mainly anthocyanin and flavanols exclusively present in their glycosylated forms (Cho et al., 2004). The main anthocyanins in fruits are glycosides of six anthocyanidins, with cyanidin as the predominant anthocyanidin, followed by delphinidin, peonidin, pelargonidin, petunidin and malvidin (Moyer et al., 2002, Stintzing et al., 2002, Prior et al., 2006). Delphinidin is known to be responsible for bluish colours, whereas cyanidin and pelargonidin are responsible for red and purple colours in the fruits and vegetables respectively. The antioxidant capacity of flavonoids is influenced by the type of sugar moiety, degree of glycosylation, and acylation of anthocyanin glucosides (Wang et al., 1997, Matsufuzi et al., 2003). HPLC analysis was performed on samples of lingonberry fruits and leaves in order to characterize the phenolic moieties present and a mass analyzer was used to determine the mass of the compounds. Samples were run through a liquid chromatographic column which separated the compounds depending on their interaction with stationary and mobile phases. The mass analyzer ionized the compounds and determined the m/z value of the ionized compounds. Compounds were identified by matching the m/z values with those reported in the literature (Sari et al., 2006, Rebecca et al., 2017). Retention times were also used to aid in the identification of compounds. Retention time varies depending on the instrument, its parameters, type and concentration of the mobile phase, but as long as the chronological order of the compounds detected matches the literature values, these compounds can be tentatively identified. The chromatograms for lingonberry fruit and leaf extract from each year are given below.

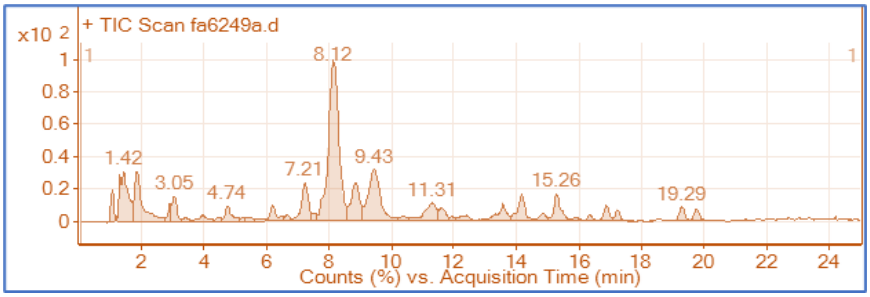

Figure 3.16: HPLC chromatogram of lingonberry fruits extract harvested in 2017.

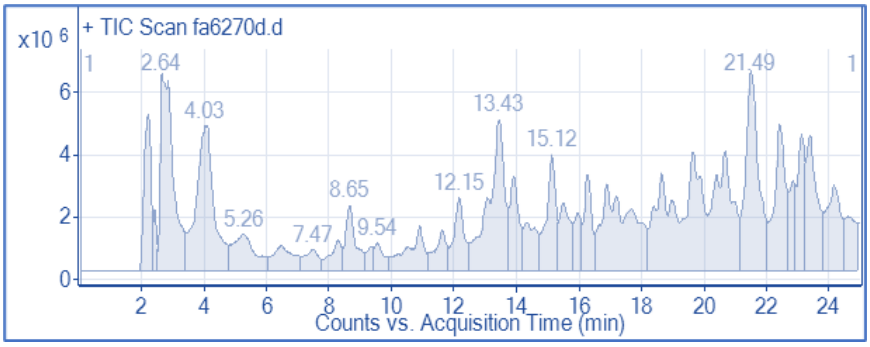

Figure 3.17: HPLC chromatogram of lingonberry leaves extract harvested in 2017.

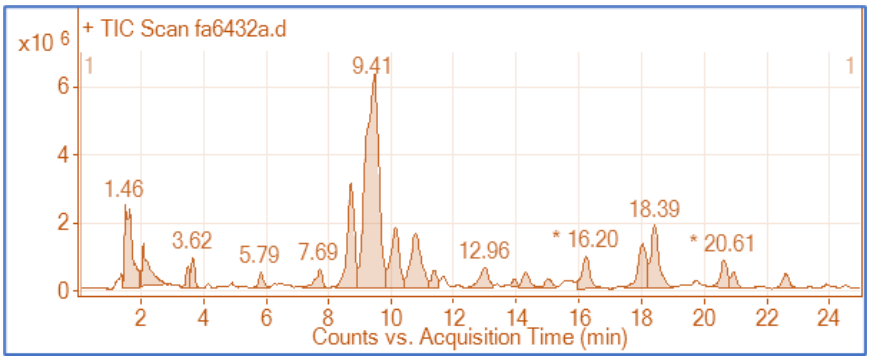

Figure 3.18: HPLC chromatogram of lingonberry fruits extract harvested in 2018.

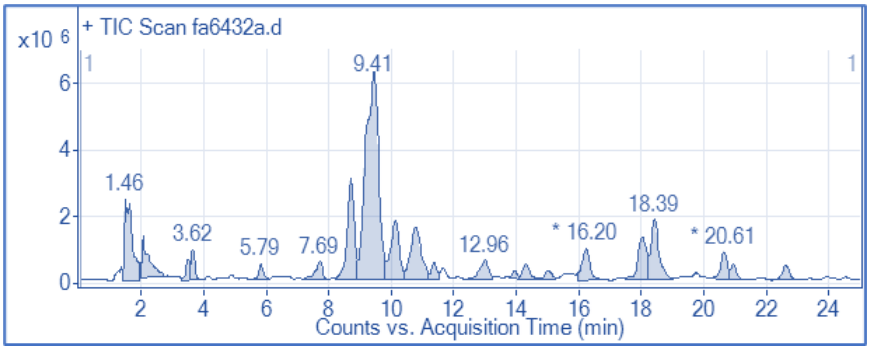

Figure 3.19: HPLC chromatogram of lingonberry leaves extract harvested in 2018.

The compounds found in the experiments are listed in Tables 3.1 through 3.4 according to their retention times. The fruits were found to have more phenolic compounds than the leaves. A total of 24 compounds from fruits and 18 compounds were tentatively identified from the leaves sample collected in 2017. On the other hand, a total of 25 compounds have been tentatively identified from the lingonberry fruits and 23 compounds from leaf samples collected in 2018.

Table 3.1: A list of tentatively identified compounds of lingonberry fruits from Lion's Den in 2017 using HPLC- TOF mass analyzer.

No of the compounds	Retention time (R_t min)	Tentatively identified compounds	m/z value	m/z value from literature
1	5.25	proanthocyanidin A	579.15	579
2	6.03	proanthocyanidin B	579.15	579
3	6.81	catechin	291.09	291
4	7.23	proanthocyanidin B	579.15	579
5	8.10	cyanidin 3-galactoside	449.11	449
6	9.63	epicatechin	291.08	291
7	10.23	cyanidin 3-glucoside	449.10	449
8	11.39	cyanidin 3-arabinoside	419.10	419
9	12.09	proanthocyanidin A	577.13	577
10	13.20	quercetin 3-O-β galactoside	465.14	465
11	13.98	ferulic acid hexoside	357.12	357
12	14.91	kaempferol deoxyhexoside	432.19	432
13	15.57	2" caffeoyl arbutin	435.16	435
14	15.74	ferulic acid hexoside	357.19	357
15	16.13	kaempferol pentoside	419.18	419
16	16.52	caffeoyl hexose hydroxyphenol	435.13	435
17	16.55	coumaroyl hexose hydroxyphenol	419.19	419
18	16.89	quercetin 3-glucoside	465.10	465
19	17.23	quercetin O (hexose deoxy hexoside)	611.16	611
20	17.56	quercetin 3-O-β xyloside	435.09	435
21	18.06	quercetin 3-O-α- arabinoside	435.09	435
22	18.63	quercetin 3-O-α arabinofuranoside	435.09	435
23	19.77	quercetin 3 -O- α rhamnoside	449.11	449
24	20.88	quercetin 3- O-(1" HMG) α rhamnoside	593.15	593

Table 3.2: A list of tentatively identified compounds of lingonberry leaves from Lion's Den in 2017 using HPLC-TOF mass analyzer.

No of the compounds	Retention time (R_t min)	Tentatively identified compounds	m/z value	m/z value from literature
1	4.93	2" caffeoyl arbutin	435.15	435
2	5.13	Quercetin 3-O-β galactoside	465.14	465
3	5.33	cyanidin 3-glucoside	449.10	449
4	6.09	cyanidin 3-arabinoside	419.15	419
5	6.21	caffeoyl hexose hydroxyphenol	435.13	435
6	7.62	kaempferol pentoside	419.15	419
7	8.12	Cyanidin 3-galactoside	449.17	449
8	8.40	coumaroyl hexose hydroxyphenol	419.19	419
9	18.52	Catechin	291.09	291
10	19.18	quercetin 3 O-(1" HMG) α rhamnoside	593.22	593
11	21.31	ferulic acid hexoside	357.19	357
12	21.44	quercetin 3-glucoside	465.22	465
13	21.57	ferulic acid hexoside	357.19	357
14	21.99	quercetin O (hexose deoxy hexoside)	611.16	611
15	22.42	proanthocyanidin A	577.23	577
16	22.73	Quercetin 3-O-β xyloside	435.09	435
17	23.03	Quercetin 3-O-α arabinoside	435.10	435
18	24.89	Quercetin 3-O-α rhamnoside	449.11	449

Table 3.3: A list of tentatively identified compounds of lingonberry fruit from Simm's beach in 2018 using HPLC-TOF mass analyzer.

No of the compounds	Retention time (R_t min)	Tentatively identified compounds	m/z value	m/z value from literature
1	5.61	proanthocyanidin B	579.14	579
2	8.45	cyanidin 3-galactoside	449.11	449
3	10.39	catechin	291.08	291
4	11.19	proanthocyanidin B	579.14	579
5	14.06	proanthocyanidin A	577.13	577
6	17.68	kaempferol deoxy hexoside	432.17	432
7	20.36	caffeoyl hexose hydroxyphenol	435.09	435
8	20.58	cyanidin 3-glucoside	449.11	449
9	20.78	proanthocyanin A	577.23	577
10	34.63	2" caffeoyl arbutin	435.17	435
11	35.36	proanthocyanidin B	579.15	579
12	37.38	cyanidin 3-arabinoside	419.15	419
13	42.07	coumaroyl hexose hydroxyphenol	419.19	419
14	42.67	quercetin 3-O-β galactoside	465.10	465
15	43.85	coumaroyl hexose hydroxyphenol	419.19	419
16	44.09	caffeoyl hexose hydroxyphenol	435.09	435
17	44.32	quercetin 3-O-glucoside	465.10	465
18	44.37	kaempferol pentoside	419.19	419
19	44.78	quercetin 3-O-β xyloside	435.09	435
20	45.25	quercetin 3-O-α arabinoside	435.09	435
21	45.65	kaemferol (HMG) rhamnoside	577.23	577
22	45.66	quercetin 3-O-α arabinofuranoside	435.09	435
23	45.98	quercetin 3-O-α rhamnoside	449.11	449
24	48.58	quercetin 3-O-(4" HMG) α rhamnoside	593.15	593
25	48.91	quercetin O (hexose- deoxyhexoside)	611.25	611

Table 3.4: A list of tentatively identified compounds of lingonberry leaves from Simm's Beach in 2018 using HPLC-TOF mass analyzer.

No of the compounds	Retention time (R_t min)	Tentatively identified compounds	m/z value	m/z value from literature
1	5.15	proanthocyanidin B	579.14	579
2	8.33	cyanidin 3-galactoside	449.11	449
3	10.08	catechin	291.08	291
4	11.19	proanthocyanidin B	579.14	579
5	14.34	proanthocyanidin A	577.13	577
6	20.40	caffeoyl hexose hydroxyphenol	435.09	435
7	20.93	cyanidin 3-glucoside	449.11	449
8	20.99	proanthocyanin A	577.23	577
9	33.57	2" caffeoyl arbutin	435.34	435
10	34.25	proanthocyanidin B	579.13	579
11	37.22	cyanidin 3-arabinoside	419.15	419
12	42.53	coumaroyl hexose hydroxyphenol	419.10	419
13	42.92	quercetin 3-O-β galactoside	465.11	465
14	42.99	coumaroyl hexose hydroxyphenol	419.09	419
15	43.32	caffeoyl hexose hydroxyphenol	435.10	435
16	43.42	quercetin 3-O-glucoside	465.14	465
17	43.82	kaempferol pentoside	419.10	419
18	44.78	quercetin 3-O-β xyloside	435.09	435
19	43.92	quercetin 3-O-α arabinoside	435.09	435
20	44.32	kaemferol (HMG) rhamnoside	577.23	577
21	45.25	quercetin 3-O-α arabinofuranoside	435.09	435
22	48.35	quercetin 3- O-(4" HMG) α rhamnoside	593.15	593
23	48.80	quercetin O (hexose- deoxyhexoside)	611.16	611

All compounds from all tables above have been tentatively identified according to the comparative m/z values from Sari *et al*. (2006) and Rebecca *et al*. (2017).

3.3 Quantitative analysis using HPLC

To ensure the presence and amount of any compound in a sample both qualitative and quantitative analysis should be performed. Usually, to quantify any amount of compound, a standard of the same compound is used with which the amount can be compared. Based on previous literature, an anthocyanin named cyanidin 3- galactoside is the major bioactive compound in lingonberries (Lehtonen et al., 2009). In this study, the amount of cyanidin 3-galactoside was determined in lingonberry fruits and leaves from 2017 and 2018.

In order to quantify the amount of cyanidin 3-galactoside present in samples, commercially available cyanidin 3-galactoside was used as standard. Experiments were conducted using a previously reported method (Hossain et al., 2016). A stock solution (200 µg/ml) was made by dissolving 2.0 mg of standard powder in 10 ml of 3% formic acid. A serial dilution was conducted in order to obtain concentrations of 150 µg/mL, 100 µg/ml, 50 µg/ml and 25 µg/ml. Each standard solution was injected separately in the LC-MS. Readings of retention time (R_t) and area of the peak were taken under the same HPLC conditions of the samples in order to generate calibration curves for reference compounds. All readings were taken three times to calculate SD. The % RSD value for all five concentrations was well below 5%. The average values of all five peaks were plotted against the five concentrations and a linear equation was obtained with an R^2 value of 0.983. This linear equation has been used to determine the amount of cyanidin 3-galactoside in the samples.

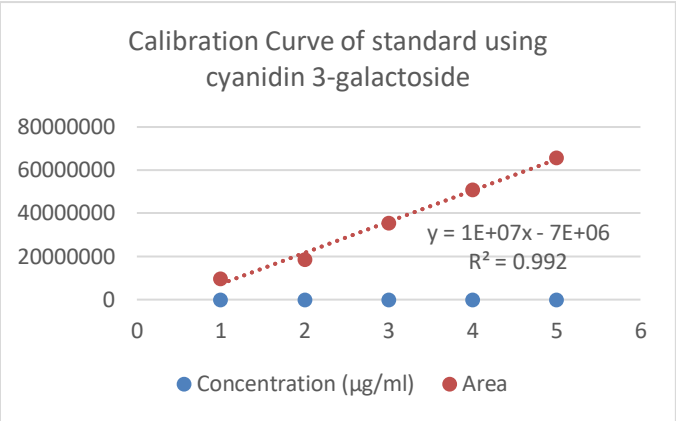

Figure 3.20: Calibration curve of standard using cyanidin 3-galactoside.

The results showed that the sample from 2017 had a higher cyanidin 3-galactoside content than the sample from 2018 and fruits contain more anthocyanin than leaves, which comply with the data of total athocyanin content from biochemical assays of lingonberry samples. Fruits had a higher amount of this particular anthocyanin, which aligns with the total anthocyanin test as fruits were richer in anthocyanin than leaves.

Table 3.5: Amount of cyanidin 3- galactoside detected in lingonberry extracts from Lion's Den in 2017 using mass analyzer.

Sample from Lion's Den in 2017	**Retention time (R_t min)**	**Compound**	**Concentration in injected sample µg/ml (determined at 520 nm)**	**Amount of cyanidin 3-galactoside per 100 gram berry fruit weight (mg/100g)**	**Percentage of cyanidin 3-galactoside in sample**
Ligonberry fruits	8.10	cyanidin 3-galactoside	621.78	248.4	0.25%
Lingonberry leaves	8.12	cyanidin 3-galactoside	281.82	112.4	0.12%

Table 3.6: Amount of cyanidin 3- galactoside detected in lingonberry extracts from Simm's Beach in 2018 using mass analyzer.

Sample from Simm's Beach in 2018	Retention time (R_t)	Compound	Concentration in injected sample µg/ml (determined at 520 nm)	Amount of cyanidin 3-galactoside per 100-gram berry fruit weight (mg/100g)	Percentage of cyanidin 3-galactoside in sample
Ligonberry fruits	8.45	cyanidin 3-galactoside	542.95	217.18	0.22%
Lingonberry leaves	8.33	cyanidin 3-galactoside	278.26	111.31	0.11%

Chapter 4: Discussion

The objective of this research was to conduct comparative analysis of various berries collected from Fogo Island and a detailed chemical analysis of polyphenols in lingonberry fruits and leaves from different locations on Fogo Island. Berries were collected for two consecutive years including blueberries, lingonberries, crowberries and bunchberries from different locations on the Island. Samples have been extracted and subjected to different chemical analysis and biochemical studies. We established a comparative statistical analysis of biochemical assays of four local berries and chemical analysis of lingonberries at the end of this research and also developed a gradient method for isolating compounds from lingonberry samples using a HPLC-MS system.

Leaf and fruit samples from each of the species of berries have been subjected to biochemical assays to determine and compare their phenolic contents. It was found that blueberries have the highest amount of phenolics among all of the samples, which is consistent with a previous study in our lab (Hossain *et al*., 2016). Phenolic amounts from blueberry leaves were statistically different from 2017 and 2018 but the same was not observed with blueberry fruits. Lingonberries have higher phenolic contents than crowberries and bunchberries and the difference between phenolic content from each leaf sample of lingonberry was statistically significant. Blueberries were also rich in flavonoids compared to other berries. After blueberries, lingonberries contain more flavonoids than crowberries and bunchberries. In general, leaves had higher flavonoid content than fruits, which is similar to previous studies in the lab (Vyas *et al*., 2013, Debnath-Canning *et al*., 2020). None of the differences in samples were statistically significant except leaves of crowberries. In the case of anthocyanin and tannin content determination, crowberries had the highest amount of respective polyphenols. Leaves had higher

anthocyanin content than fruits and samples from each year showed no statistical significance in total anthocyanin content determination except crowberry fruit samples from each year. In both total anthocyanin and tannin content assays fruits contain more than leaves. Crowberry fruit samples from 2017 had the highest tannin content and fruits of the same berry from each year were statistically significant. All leaf samples in the total tannin assay were statistically significant except for lingonberries.

To get more detailed results we collected various berry samples from different locations on Fogo Island and used the mean value to display in bar diagrams. Where each value has been plotted as mean ± SE. Not only did we collect from different locations, but we collected samples throughout two consecutive years to acquire consistency and observe whether there are any significant changes occurring between the time-lapse of one year. For most of the results, there were no significant changes for the results from two years as there were few statistical differences. Again, there were some samples which showed variation in the data for two years even when the samples have been collected and tested following the same method. For example, in the TPC test, blueberry, lingonberry and bunchberry fruits samples from two years have not shown any significant differences in their result as the differences were not statistically significant. On the other hand, all leaf samples of each berry from each year showed different results as the differences among their two years' result were statistically significant. We analyzed all of the results statistically where in the bar diagrams the same column with a different letter and number indicate statistical significance at $P< 0.05$. According to this analysis, in TFC, the differences between the two years of each sample did not show any statistical significance as $P<0.05$ except for crowberry fruits. In the total anthocyanin test fruit samples of three berries vary in time-lapse of one year as differences between their data were statistically significant,

where leaf samples have not shown any statistical significance. Blueberry, lingonberry and bunchberry fruits and leaves from each year have not shown much variation in the total tannin assay as there were no statistical significances. Other samples were statistically different except lingonberry. One reason for variation in the number of different polyphenols in berry samples might be slight degradation over the period of one year. Another reason might be genotype. Other than that, the concentration of bioactive compounds depends on climate of that location, pH of the soil, water scarcity and exposure to sunlight (Krüger *et al*., 2014, Barnuud *et al*., 2013). The weather archive of Fogo Island showed a 2° elevation of temperature in 2018 compared to 2017, which might affect the final nutrient contents in berries (Weather archive of Fogo Island). One study showed that a slight elevation in temperature can accelerate the maturation of berries and manipulate further total nutrient content (Pestore *et al*., 2017), which is also reflected in our present study. In the TPC assay, data with statistical significance showed that, samples from 2018 have higher phenolic content than samples from 2017. Here the slight increase in temperature may have affected berry production in 2018.

Berries have been tested to assay their antioxidant ability in terms of neutralizing DPPH free radicals where blueberries have the highest reducing capacity compared to lingonberries and crowberries. Leaves have shown higher antioxidant activities than fruits. This might be due to the presence of higher phenolic contents in leaves than fruits which is also consistent with previous findings in our lab (Vyas *et al*., 2013). In the DPPH free radical scavenging bioassay each sample over the time lapse of one year have shown similar results as there were no statistical significances except for lingonberry fruits, which results from two years varied as they were statistically significant. After the general comparison of blueberries, lingonberries crowberries and bunchberries in terms of biochemical activity and phenolic content we further compared the

lingonberry data for each lingonberry sample that had been collected from different locations on Fogo Island for two years, 2017 and 2018. Samples from one year showed higher phenolic content than the other. Also, samples from different locations sometimes showed significant differences or similarities. The variation in results might be due to the weather of that particular year, sunlight exposure, temperature, soil, agronomic conditions and the genotype of the sample from that particular location. As earlier mentioned, the temperature in 2018 was slightly elevated compared to 2017, which might contribute to the higher number of polyphenols in 2018 in lingonberry samples. Sometimes genotype can influence the presence of antioxidants compounds, and this factor could vary based on the location where the sample has been collected from (Hosseinian *et al*., 2007). Previous research has shown that when the same cultivars produce blueberries at different locations, and different cultivars produce blueberries at the same location, it does not have the same outcome (Connors *et al*., 2002). The antioxidant abilities of samples varied significantly which suggests that the presence of bioactive molecules varied due to the genotype and environmental factors (Connors *et al*., 2002). The outcome of our results also supports this. Besides genotype and environmental factors here, the results over the time-lapse of one year varied. The reason could be due to the changes in climate over the years, however we stored one set of samples collected in 2017 for a year and samples collected in 2018 were fresher when we tested compared to the previous years' samples, which could affect polyphenol levels. As an example, the phenolic content of lingonberry has been found higher in samples collected in 2018 and for TPC lingonberry fruits collected from Simm's Beach and leaves from Island Harbour have the highest phenolic content. However, we also tested some blueberry samples from 2017 again after approximately one year in the freezer and overall polyphenol content only decreased by ~2% (Unpublished data), so these compounds are quite stable.

After determining the amount of different phenolic compounds in fruit and leaf samples, we next characterized the compounds that lingonberry samples contain. Previously in our lab blueberry samples have been chemically analyzed in detail (Debnath-canning *et al.,* 2020). Also, commercial extracts of different berries have been analyzed in our lab in previous years (Hossain *et al*., 2016). So here we concentrated on lingonberry samples, as after blueberry lingonberry has been found to have higher antioxidant capacity and phenolic content. Samples which gave the highest antioxidant activity were chosen for further chemical analysis. Thus, the sample collected from Simm's Beach was selected for 2018 and the sample from Joe Batt's pt. Trail have been selected from 2017 for further chemical investigation. To isolate the compounds, we used HPLC and to characterize the tentative compound we used mass ionization technique. In HPLC we used different solvents for the mobile phase. The ratio of solvents was changed until we found the desired separation. Thus, we developed a gradient method to separate lingonberry fruits and leaves. HPLC-MS conditions remained the same for all of the analysis. We tentatively identified 25 and 23 compounds from lingonberry fruits and leaves samples respectively, which have been collected in 2018. Also, we tentatively identified 24 and 18 compounds from fruits and leaves sample accordingly which have been collected in 2017. In one of very few similar studies, lingonberries growing in Finland were found to have, 28 compounds identified in methanolic extracts (Sari *et al*., 2006). Also previously in our lab, research conducted on different berries identified only three compounds from a commercial extract of lingonberries (Hossain *et al*., 2016). The reason for the differences of identified compounds might be that during the present study we used the natural extracts instead of commercially available samples and used advanced equipment like the MS-TOF analyzer, which is more advanced than the analyzer which was previously used.

After the initial analysis, we proceeded to a more detailed quantitative analysis. We used a standard compound to quantify anthocyanins, which are unstable compounds. These are not readily available, and the price is very high. To determine the level of anthocyanin in glycoside form, it is very difficult to obtain a standard for every anthocyanin present in samples. For these reasons, we only ran cyanidin 3–galactoside as a standard since it was available at that time and also it has been reported to have high antioxidant capacity (Kahkonen *et al*., 2003). Thus, we determined the percentage of this particular anthocyanin in fruit and leaf samples collected from both 2017 and 2018. It has been revealed that fruits have a slightly higher amount of cyanidin 3–galactoside than leaves. Also, samples collected in the year 2018 have higher cyanidin 3–galactoside levels than samples from 2017. Again, it might be due to the variation in climate and environment over the year that affects the presence of anthocyanin in lingonberries. We have found that a sample from 2018 has 0.22% and 0.21% of cyanidin 3- galactoside in fruits and leaves respectively. Also 0.13% and 0.12% cyanidin 3- galactoside was detected in fruits and leaves samples collected in 2017. All of the amounts are higher than the amount of the same compound found in blueberries in our lab that have been collected from the other parts of Newfoundland. One reason might be that the Fogo Island climate is slightly different than the rest of Newfoundland. From the weather data in Canada, we found that the average temperature in the Fall on Fogo Island is slightly higher than in the rest of Newfoundland, which might increase the amount of certain polyphenols in berries (Pestore *et al*., 2017). Considering data from our research, Fogo Island berries might be preferable to other berries.

One of the big limitations of this study is that we have not tested these specific samples in any animal model or cell culture model. However, some of our previous lab members have tested lingonberry samples on cell cultures to determine neuroprotective effects of lingonberries

(Hossain *et al*., 2016). In that experiment, commercial samples provided complete protection against cell damage in an *in vitro* cell injury model. Application of lingonberry samples 15 minutes prior to injury provided complete protection against injury. More recent data in the lab showed protective effects of lingonberry extracts against neuroinflammation in cells (unpublished data). The reason behind the neuroprotection is likely the presence of different types of polyphenols in lingonberries samples. Different types of polyphenols exhibit various different mechanisms to give protection (Basli *et al.,* 2012), such as flavonols are brain permeable and metabolize glucuronic acid to give neuroprotection (Faria *et al.,* 2004). Catechin provides neuroprotection by inhibiting endogenous neurotoxins that can further delay the onset of Parkinson's disease (Vauzour *et al*., 2008). Catechins derivatives can also delay the onset of neurodegerative disorders like Alzheimer's disease by inhibiting mediators responsible for apoptotic cell death (Li *et al*., 2004). Other mechanisms involve iron chelation, neutralizing free radicals and modulating pro-survival genes (Mandel *et al*., 2004). Proanthocyanidin and tannins exert their neuroprotective nature by interacting with specific neurons (Narita *et al.,* 2011). Studies have shown that proanthocyanidin and condensed tannins prevent cardiovascular diseases and cancer and give protection against age related oxidative brain damage (Sato *et al*., 1999, Aldini *et al*., 2003, Deshane *et al*., 2004). As strong antioxidants, anthocyanins provide protection against age-related oxidative stress thus preventing DNA fragmentation and lipid peroxidation (Acquaviva *et al*., 2003, Di Giacomo *et al*., 2007). The present study showed the presence of several polyphenols such as flavonols, catechin derivatives, anthocyanins, proanthocyanidin and tannins. Also, cyanidin 3-galactoside is a major anthocyanin that has been found in a noticeable amount in lingonberry from this present study, which indicates that the lingonberry fruits might be a potential drug candidate for neurodegenerative disorders such as

Parkinson's disease, Alzheimer's disease, motor neuron disease, Huntington's disease and spinal muscular atrophy.

Another drawback of our present study is that we have not done a bioavailability study to determine the presence of polyphenols in animal organs, but as an extension of this present study, we have fed mice lingonberry fruits and leaf samples and then preserved part of their brain tissue to later determine the bioavailability of polyphenols in the brain. To exert neuroprotective activity, polyphenols have to cross the blood-brain barrier (BBB). Though present research in *in vitro* studies of lingonberries shows promise, several studies showed that the same samples might have different *in vivo* results because of metabolization of compounds inside the body (Espín *et al*., 2007). Again, other studies have shown compounds can cross the BBB, which increases the interest to research these berries even further (Andrade *et al*., 2018, Jung *et al*., 2018). Another study revealed that phenolic sulphates produced after colonic metabolism can reach the brain by crossing the endothelial cells of the BBB and can modulate microglia-mediated inflammation and exert neuroprotective effects (Figueira *et al.,* 2017). Along with neuroprotection, lingonberries have some other benefits over other berries that can make it a super berry of this century such as improving gut health (Heyman-Lindén *et al*., 2016), combating obesity (Ryyti *et al*., 2020), slowing the aging process (Shammas *et al.,* 2011) and treating UTIs (Kontiokari *et al.,* 2001).

The growing health benefits of lingonberry make it increasingly popular in North America, although it is a lesser-known berry in this territory (Burt and Penhallegon, 2003, Economic Evaluation of Lingonberry Production in Oregon). Commercially, lingonberries are available now in powder (product name: arctic Powder), capsule (product name: Lingonberry Dietary supplement Cranberry Capsule Food) and liquid (as juice) forms (Dryck Lingon by Ikea). Although people are commercially producing lingonberries and eating them raw or cooked there

are not many commercially available supplements that are currently available. In our present research we have found that lingonberries have high tannin and anthocyanin levels and considering the health benefits of polyphenols it might be a potential drug or nutraceutical candidate used to treat neurodegenerative disorders or can be used as dietary supplements.

In a fast-changing world where climate and the environment are being altered drastically, pollution is increasing more than ever, food habits are shifting in an unhealthy way, and stress is releasing free radicals in our bodies continuously, we need to find an option that can reduce the oxidative stress in our body along with adopting other healthier habits. Considering the health benefits of lingonberries, the berries themselves or supplements made of lingonberries can be a viable option for all of us.

Chapter 5: Conclusions and future directions

In this study, the phenolic contents of blueberries, lingonberries, crowberries and bunchberries native to Fogo Island were determined and compared. The results showed that blueberries have a higher amount of phenolic content in comparison to the other berries. It was also found that blueberry leaves contain higher amounts of phenolic content than blueberry fruits. There has been a lot of research conducted on blueberries. However, research on lingonberry is rather limited, although it has been shown to have antioxidant properties similar to blueberries (Adams et al., 2011). In this research project, the chemical composition of lingonberry fruits and leaves was studied. A total of 25 compounds including flavonoids, anthocyanins and various glycosides were identified from lingonberry fruit and leaf samples using HPLC-MS. One anthocyanin named cyanidin 3-galactoside has been quantified in fruits and leaves. It was found that statistically, lingonberry fruits have higher amounts of anthocyanins than lingonberry leaves, which is consistent with other berry species.

In this investigation, cyanidin 3-galactoside was the only standard that we used for quantification, mainly due to the lack of availability of other standards and cost. Also, it was known to be the major anthocyanin in berries. There are several other anthocyanins and bioactive molecules in lingonberry, which still need to be quantified. These lingonberry extracts need to be further tested in other studies such as toxicological and bioavailability studies. As examples, samples can be tested in *in vitro* models of neurodegeneration and in *in vivo* diet studies in rodents. Based on more biological studies and the existing data on the neuroprotective effects of lingonberries, the development of dietary supplements or nutraceutical products could not only be beneficial for health but may also be of economic benefit to Fogo Island, and to Newfoundland and Labrador as a whole.

References

Abbott, N. J., Patabendige, A. A., Dolman, D. E., Yusof, S. R., & Begley, D. J., (2010). Structure and function of the blood-brain barrier. *Neurobiology of disease*, *37*(1), 13–25. doi.org/10.1016/j.nbd.2009.07.030

Abourashed E. A., (2013). Bioavailability of Plant-Derived Antioxidants. *Antioxidants (Basel, Switzerland)*, *2*(4), 309–325. doi.org/10.3390/antiox2040309

Aggarwal, B. B., & Shishodia, S. (2006). Molecular targets of dietary agents for prevention and therapy of cancer. *Biochemical pharmacology*, *71*(10), 1397–1421. doi.org/10.1016/j.bcp.2006.02.009

Al-Badr, A., & Al-Shaikh, G. (2013). Recurrent Urinary Tract Infections Management in Women: A review. *Sultan Qaboos University medical journal*, *13*(3), 359–367. doi.org/10.12816/0003256

Alzheimer's Association (2008). 2008 Alzheimer's disease facts and figures. *Alzheimer's & dementia: the journal of the Alzheimer's Association*, *4*(2), 110–133. doi.org/10.1016/j.jalz.2008.02.005

Anderson, O. M., & Jordheim, M. (2006). *The anthocyanins. In: Anderson O.M., Markham K.R., editors. Flavonoids: Chemistry, Biochemistry and Applications.* CRC Press/Taylor & Francis Group; Boca Raton, FL, USA.

Antolak, H., Oracz, J., Otlewska, A., Żyżelewicz, D., & Kręgiel, D. (2017). Identification of Carotenoids and Isoprenoid Quinones from Asaia lannensis and Asaia bogorensis. *Molecules (Basel, Switzerland)*, *22*(10), 1608. doi.org/10.3390/molecules22101608

Backhed, F., Wang, T., Hooper, L. V., Koh, G. Y & Nagy, A. (2004). The gut microbiota as an environmental factor that regulates fat storage. The proceedings of the national academy of sciences, 101(44), 15718–23, doi: 10.1073/pnas.0407076101

Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J. S., Lopez-Lluch, G., Lewis, K., Pistell, P. J., Poosala, S., Becker, K. G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K. W., Spencer, R. G., Lakatta, E. G & Sinclair, D. A. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*, *444*(7117), 337–342. doi.org/10.1038/nature05354

Beattie, J., Crozier, A., & Duthie, G. D. (2005). Potential Health Benefits of Berries. Current Nutrition & Food Science, 1, 71-86. doi:10.2174/1573401052953294

Bhullar, K. S., & Hubbard, B. P. (2015). Lifespan and healthspan extension by resveratrol. *Biochimica et biophysica acta*, *1852*(6), 1209–1218. doi.org/10.1016/j.bbadis.2015.01.012

Boland T. (2010). *Wildflowers of Fogo Island and Change Islands.* Key Porter Books Limited, Toronto, Ont.

Burt L. A., & Penhallogen R. (2003). *Economic evaluation of lingonberry production in Oregon*, Oregon State University Extension Service.

Chan, R. C., Reid, G., Irvin, R. T., Bruce, A. W., & Costerton, J. W. (1985). Competitive exclusion of uropathogens from human uroepithelial cells by Lactobacillus whole cells and cell wall fragments. *Infection and immunity*, *47*(1), 84–89. doi.org/10.1128/iai.47.1.84-89.1985

Chandrasekara, N., & Shahidi, F. (2011). Effect of roasting on phenolic content and antioxidant activities of whole cashew nuts, kernels, and testa. *Journal of agricultural and food chemistry*, *59*(9), 5006–5014. doi.org/10.1021/jf2000772

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., & Zhao, L. (2017). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, *9*(6), 7204–7218. doi.org/10.18632/oncotarget.23208

Chen, Q. Y., Alarcon, S., Tharp, A., Ahmed, O. M., Estrella, N. L., Greene, T. A., Rucker, J., & Breslin, P. A. (2009). Perceptual variation in umami taste and polymorphisms in TAS1R taste receptor genes. *The American journal of clinical nutrition*, *90*(3), 770S–779S. doi.org/10.3945/ajcn.2009.27462N

Cherubini, A., Beal, M. F., & Frei, B. (1999). Black tea increases the resistance of human plasma to lipid peroxidation in vitro, but not ex vivo. *Free radical biology & medicine*, *27*(3-4), 381–387. doi.org/10.1016/s0891-5849(99)00064-7

Cheynier V. (2005). Polyphenols in foods are more complex than often thought. *The American journal of clinical nutrition*, *81*(1 Suppl), 223S–229S. doi.org/10.1093/ajcn/81.1.223S

Cho, M.J., Howard, L.R., Prior, R.L., & Clark, J.R. (2004). Flavonoid glycosides and antioxidant capacity of various black berry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *Journal of the Science of Food and Agriculture, 84(13),* 1771-1782. doi: 10.1002/jsfa.1885.

Chun, O. K., Chung, S. J., Claycombe, K. J., & Song, W. O. (2008). Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in U.S. adults. *The Journal of nutrition*, *138*(4), 753–760. doi.org/10.1093/jn/138.4.753

Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (1998). Tannins and human health: a review. *Critical reviews in food science and nutrition*, *38*(6), 421–464. doi.org/10.1080/10408699891274273

Connor, A. M., Luby, J. J., Tong, C. B., Finn, C. E., & Hancock, J. F. (2002). Genotypic and Environmental Variation in Antioxidant Activity, Total Phenolic Content, and Anthocyanin Content among Blueberry Cultivars, *Journal of the American Society for Horticultural Science jashs*, *127*(1), 89-97.

Corley, J., Kyle, J. A., Starr, J. M., McNeill, G., & Deary, I. J. (2015). Dietary factors and biomarkers of systemic inflammation in older people: the Lothian Birth Cohort 1936. *The British journal of nutrition*, *114*(7), 1088–1098. doi.org/10.1017/S000711451500210X

Crespy, V., Morand, C., Besson, C., Manach, C., Demigne, C., & Remesy, C. (2002). Quercetin, but not its glycosides, is absorbed from the rat stomach. *Journal of agricultural and food chemistry*, *50*(3), 618–621. doi.org/10.1021/jf010919h

Das, K & Roychoudhury. (2014). A review article-Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontier in Environmental Science.* doi.org/10.3389/fenvs.2014.00053.

De Calignon, A., Polydoro, M., Suárez-Calvet, M., William, C., Adamowicz, D. H., Kopeikina, K. J., Pitstick, R., Sahara, N., Ashe, K. H., Carlson, G. A., Spires-Jones, T. L., & Hyman, B. T. (2012). Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron*, *73*(4), 685–697. https://doi.org/10.1016/j.neuron.2011.11.033.

de Souza, V. R., Pereira, P. A., da Silva, T. L., de Oliveira Lima, L. C., Pio, R., & Queiroz, F. (2014). Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. *Food chemistry*, *156*, 362–368. doi.org/10.1016/j.foodchem.2014.01.125

Debnath, S. C. (2017). Temporary immersion and stationary bioreactors for mass propagation of true-to-type highbush, half-high, and hybrid blueberries (Vaccinium spp.). *The Journal of Horticultural Science and Biotechnology, 92(1),* 72-80. doi: 10.1080/14620316.2016.1224606

Debnath-Canning, M., Unruh, S., Vyas, P., Daneshtaleb, N., Igamberdiev, A. U., & Weber, J. T. (2020). Fruits and leaves from wild blueberry plants contain diverse polyphenols and decrease neuroinflammatory responses in microglia. Journal of Functional Foods, 68. doi.org/10.1016/j.jff.2020.103906

Demmig-Adams, B., & Adams, W. W., 3rd (2002). Antioxidants in photosynthesis and human nutrition. *Science (New York, N.Y.)*, *298*(5601), 2149–2153. doi.org/10.1126/science.1078002

Deprez, S., Mila, I., Huneau, J. F., Tome, D., & Scalbert, A. (2001). Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. *Antioxidants & redox signaling*, *3*(6), 957–967. doi.org/10.1089/152308601317203503

Di Meo, S., Reed, T. T., Venditti, P., & Victor, V. M. (2016). Harmful and Beneficial Role of ROS. *Oxidative medicine and cellular longevity*, *2016*, 7909186. https://doi.org/10.1155/2016/7909186

Dias, M. C., Pinto, D. C. G. A., & Silva, A. M. S. (2021). Plant Flavonoids: Chemical Characteristics and Biological Activity. *Molecules (Basel, Switzerland)*, *26*(17), 5377. https://doi.org/10.3390/molecules26175377

Ding, S., Chi, M. M., Scull, B. P., Rigby, R., Schwerbrock, N. M., Magness, S., Jobin, C., & Lund, P. K. (2010). High-fat diet: bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. *PloS one*, *5*(8), e12191. doi.org/10.1371/journal.pone.0012191

Dong, X. X., Wang, Y., & Qin, Z. H. (2009). Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacologica Sinica, 30(4)*, 379-387. doi: 10.1038/aps.2009.24.

Dong, Z., Surh, Y. J., Packer, L., & Cadenas, E. (2009). *Dietary Modulation of Cell Signaling Pathways.* CRC Press. FL, USA.

Dröge W. (2002). Free radicals in the physiological control of cell function. *Physiological reviews*, *82*(1), 47–95. doi.org/10.1152/physrev.00018.2001

Dróżdż, P., Šėžienė, V., & Pyrzynska, K. (2017). Phytochemical Properties and Antioxidant Activities of Extracts from Wild Blueberries and Lingonberries. *Plant foods for human nutrition (Dordrecht, Netherlands)*, *72*(4), 360–364. doi.org/10.1007/s11130-017-0640-3

Dróżdż, P., Šėžienė, V., & Pyrzynska, K. (2017). Phytochemical Properties and Antioxidant Activities of Extracts from Wild Blueberries and Lingonberries. *Plant foods for human nutrition (Dordrecht, Netherlands)*, *72*(4), 360–364. doi.org/10.1007/s11130-017-0640-3

Dróżdż, P., Šėžienė, V., Wójcik, J., & Pyrzyńska, K. (2017). Evaluation of Bioactive Compounds, Minerals and Antioxidant Activity of Lingonberry (Vaccinium vitis-idaea L.) Fruits. *Molecules (Basel, Switzerland)*, *23*(1), 53. doi.org/10.3390/molecules23010053

Duke, J. A., & Ayensu. E. S. (1985). *Medicinal Plants of China.* Reference Publications, Inc.

Duthie, G., G., & Brown, K., M. (1994). *Reducing the risk of cardiovascular disease. In: Goldberg I., editor. Functional Foods: Designer Foods, Pharmafoods, Nutraceuticals.* Chapman & Hall; New York, NY, USA.

Ek, S., Kartimo, H., Mattila, S., & Tolonen, A. (2006). Characterization of phenolic compounds from lingonberry (Vaccinium vitis-idaea). *Journal of agricultural and food chemistry*, *54*(26), 9834–9842. doi.org/10.1021/jf0623687

Elias, T. &., Dykeman, P. (1982). *A Field Guide to N. American Edible Wild Plants.* Sterling.

Fabbrini, E., Sullivan, S., & Klein, S. (2010). Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology (Baltimore, Md.)*, *51*(2), 679–689. doi.org/10.1002/hep.23280

Facciola. S. (1990). *Cornucopia - A Source Book of Edible Plants*. Kampong Publications.

Fan, Z. L., Wang, Z. Y., & Liu, J. R (2011). Cold-field fruit extracts exert different antioxidant and antiproliferative activities in vitro. *Food Chemistry. 129*, 402–407. doi: 10.1016/j.fodchem.2011.04.091.

Faria, A., Pestana, D., Teixeira, D., Couraud, P. O., Romero, I., Weksler, B., de Freitas, V., Mateus, N., & Calhau, C. (2011). Insights into the putative catechin and epicatechin transport across blood-brain barrier. *Food & function*, *2*(1), 39–44. doi.org/10.1039/c0fo00100g

Figueira, I., Garcia, G., Pimpão, R. C., Terrasso, A. P., Costa, I., Almeida, A. F., Tavares, L., Pais, T. F., Pinto, P., Ventura, M. R., Filipe, A., McDougall, G. J., Stewart, D., Kim, K. S., Palmela, I., Brites, D., Brito, M. A., Brito, C., & Santos, C. N. (2017). Polyphenols journey through blood-brain barrier towards neuronal protection. *Scientific reports*, *7*(1), 11456. doi.org/10.1038/s41598-017-11512-6

Fogo Island By Robin Esrock, National Geographic, retrieved from, https://www.nationalgeographic.com/travel/canada/fogo-island-newfoundland-and-labrador/#close

Foley, S. L., & Debnath, S. C. (2007). Influence of in vitro and ex vitro propagation on anthocyanin content and anti-oxidant activity of lingonberries. *Journal of Horticultural Science and Biotechnology, 82(1),* 114-118. doi:10.1080/14620316.2007.11512207.

Foster, S., & Duke, J. A. (1990). *A Field Guide to Medicinal Plants. Eastern and Central N. America*. Houghton Mifflin Co.

Fraser, M. H., Cuerrier, A. Haddad, P. S., Arnason, J. T., Owen, P. L., & Johns, T. (2007). Medicinal plants of Cree communities (Quebec, Canada): antioxidant activity of plants used to treat type 2 diabetes symptoms. *Canadian Journal of Physiology and Pharmacology. 85(11)*, 1200–1214. doi: 10.1139/Y07-108

Frei, B., Stocker, R., & Ames, B. N. (1988). Antioxidant defenses and lipid peroxidation in human blood plasma. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(24), 9748–9752. doi.org/10.1073/pnas.85.24.9748

Frost, B., & Diamond, M. I. (2010). Prion-like mechanisms in neurodegenerative diseases. *Nature reviews. Neuroscience*, *11*(3), 155–159. doi.org/10.1038/nrn2786

Galanakis, C. M., (2017). *Nutraceutical and Functional Food Components,* Academic Press.

Gee, J. M., DuPont, M. S., Rhodes, M. J., & Johnson, I. T. (1998). Quercetin glucosides interact with the intestinal glucose transport pathway. *Free radical biology & medicine*, *25*(1), 19–25. doi.org/10.1016/s0891-5849(98)00020-3

Ghayur, M. N., & Janssen, L. J. (2010). Nephroprotective drugs from traditionally used Aboriginal medicinal plants. *Kidney international*, *77*(5), 471–472. doi.org/10.1038/ki.2009.507

Gibson, G. (1998). Dietary modulation of the human gut microflora using prebiotics. *British Journal of Nutrition, 80*(S2), S209-S212. doi:10.1017/S0007114500006048

Gonçalves, A. C., Nunes, A. R., Falcão, A., Alves, G., & Silva, L. R. (2021). Dietary Effects of Anthocyanins in Human Health: A Comprehensive Review. *Pharmaceuticals (Basel, Switzerland)*, *14*(7), 690. https://doi.org/10.3390/ph14070690

Gray D. H. (2000). Discovering Rocks off Labrador: A Photo Essay. IBRU boundary and security bulletin. Retrieved from, https://www.dur.ac.uk/resources/ibru/publications/full/bsb8-2_gray.pdf

Griesbach R. (2005). Biochemistry and genetics of flower color. *Plant Breeding Reviews, 25*, 89–114.

Gucker C. L. (2012). Cornus canadensis. IN: Fischer, W.C. (compiler). The fire effects information system. United States Department of Agriculture, Forest Service, Intermountain Research Station, Intermountain Fire Sciences Laboratory, Missoula, Montana. Retrieved from, http://www.fs.fed.us/database/feis/plants/forb/corcan/i ntroductory.

Gundesli, M. A., Korkmaz, N., & Okatan, V. (2019). Polyphenol content and antioxidant capacity of berries: A review. *International Journal of Agriculture for Life Science. 3(2),* 350-361, e-ISSN: 2602-4381

Gupta, K., Scholes, D., & Stamm, W. E. (1999). Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. *JAMA*, *281*(8), 736–738. doi.org/10.1001/jama.281.8.736

Hajazimi, E., Landberg, R., & Zamaratskaia, G. (2016). Simultaneous determination of flavonols and phenolic acids by HPLC-CoulArray in berries common in the Nordic diet. *LWT—Food Sci. Technol, 74(C),* 128–134. doi: 10.1016/j.lwt.2016.07.034

Hall, I., & Sibley, J. D. (1976). The biology of Canadian weeds. *Canadian Journal of Plant Science*, 56, 885-892. doi: 10.4141/cjps76-144

Halliwell B. (1995). How to characterize an antioxidant: an update. *Biochemical Society symposium*, *61*, 73–101. doi.org/10.1042/bss0610073

Halliwell, B., Zhao, K., & Whiteman, M. (2000). The gastrointestinal tract: a major site of antioxidant action?. *Free radical research*, *33*(6), 819–830. doi.org/10.1080/10715760000301341

Halvorsen, B. L., Holte, K., Myhrstad, M. C., Barikmo, I., Hvattum, E., Remberg, S. F., Wold, A. B., Haffner, K., Baugerød, H., Andersen, L. F., Moskaug, Ø., Jacobs, D. R., Jr, & Blomhoff, R. (2002). A systematic screening of total antioxidants in dietary plants. *The Journal of nutrition*, *132*(3), 461–471. doi.org/10.1093/jn/132.3.461

Harborne, J. B & Williams, C. A. (2000). Advances in flavonoid research since 1992. Phytochemistry, 55(6):481–504. doi: 10.1016/s0031-9422(00)00235-1.

Hardman W. E. (2014). Diet components can suppress inflammation and reduce cancer risk. *Nutrition research and practice*, *8*(3), 233–240. doi.org/10.4162/nrp.2014.8.3.233

Hardy, J., & Revesz, T. (2012). The spread of neurodegenerative disease. *The New England journal of medicine*, *366*(22), 2126–2128. doi.org/10.1056/NEJMcibr1202401

Haynes, C. M., Titus, E. A., & Cooper, A. A. (2004). Degradation of misfolded proteins prevents ER-derived oxidative stress and cell death. *Molecular Cell, 15(5),* 767-776, doi: 10.1016/j.molcel.2004.08.025.

Heidenreich., C. (2010). The lowdown on lingonberries. New York Berry News, 9(6), 7-8.

Heinonen M. (2007). Antioxidant activity and antimicrobial effect of berry phenolics--a Finnish perspective. *Molecular nutrition & food research*, *51*(6), 684–691. doi.org/10.1002/mnfr.200700006

Heller C. A. (1953). *Wild edible and poisonous plants of Alaska,* Cooperative extension work in Agriculture and Home Economics Extension Service, University of Alaska.

Heyman-Lindén, L., Kotowska, D., Sand, E., Bjursell, M., Plaza, M., Turner, C., Holm, C., Fåk, F., & Berger, K. (2016). Lingonberries alter the gut microbiota and prevent low-grade inflammation in high-fat diet fed mice. *Food & nutrition research*, *60*, 29993. doi.org/10.3402/fnr.v60.29993

Hollman, P. C., & Katan, M. B. (1997). Absorption, metabolism and health effects of dietary flavonoids in man. *Biomedicine & pharmacotherapy*, *51*(8), 305–310. doi.org/10.1016/s0753-3322(97)88045-6

Hoornstra, D., Vesterlin, J., Pärnänen, P., Al-Samadi, A., Zlotogorski-Hurvitz, A., Vered, M., & Salo, T. (2018). Fermented Lingonberry Juice Inhibits Oral Tongue Squamous Cell Carcinoma Invasion *In Vitro* Similarly to Curcumin. *In vivo (Athens, Greece)*, *32*(5), 1089–1095. doi.org/10.21873/invivo.11350

Hossain, M. Z., Shea, E., Daneshtalab, M & Weber, J. T. (2016). Chemical Analysis of Extracts from Newfoundland Berries and Potential Neuroprotective Effects. *Antioxidants (Basel), 5(4),* 36. doi: 10.3390/antiox5040036.

Hosseinian, F.S., & Beta, T. (2007). Saskatoon and wild blueberries have higher anthocyanin contents than other Manitoba berries. *Journal of Agriculture and Food Chemistry, 55(26),* 10832-10838. doi: 10.1021/jf072529m.

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. Nature. 444(7121), 860–867, doi: 10.1038/nature05485

Howell, A. B., Vorsa, N., Der Marderosian, A., & Foo, L. Y. (1998). Inhibition of the adherence of P-fimbriated Escherichia coli to uroepithelial-cell surfaces by proanthocyanidin extracts from cranberries. *The New England journal of medicine*, *339*(15), 1085–1086. doi.org/10.1056/NEJM199810083391516

Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C., & Rahu, N. (2016). Oxidative Stress and Inflammation: What Polyphenols Can Do for Us?. *Oxidative medicine and cellular longevity*, *2016*, 7432797. doi.org/10.1155/2016/7432797

Hyun, T. K., Kim, H. C., Ko, Y. J., & Kim, J. S. (2016). Antioxidant, α-glucosidase inhibitory and anti-inflammatory effects of aerial parts extract from Korean crowberry (Empetrum nigrum var. japonicum). *Saudi journal of biological sciences*, *23*(2), 181–188. doi.org/10.1016/j.sjbs.2015.02.008

Ichinose, M. , Sugiura, H., Yamagata, S., Koarai, A & Shirato, K. (1999). Increase in Reactive Nitrogen Species Production in Chronic Obstructive Pulmonary Disease Airways. *American Journal of Respiratory and Critical Care Medicine*, 162(2). doi.org/10.1164/ajrccm.162.2.9908132

Jennings, A., Welch, A. A., Fairweather-Tait, S. J., Kay, C., Minihane, A. M., Chowienczyk, P., Jiang, B., Cecelja, M., Spector, T., Macgregor, A., & Cassidy, A. (2012). Higher anthocyanin intake is associated with lower arterial stiffness and central blood pressure in women. *The American journal of clinical nutrition*, *96*(4), 781–788. doi.org/10.3945/ajcn.112.042036

Jones, G. A., McAllister, T. A., Muir, A. D., & Cheng, K. J. (1994). Effects of Sainfoin (Onobrychis viciifolia Scop.) Condensed Tannins on Growth and Proteolysis by Four Strains of Ruminal Bacteria. *Applied and environmental microbiology*, *60*(4), 1374–1378. doi.org/10.1128/aem.60.4.1374-1378.1994

Joseph, S. V., Edirisinghe, I., & Burton-Freeman, B. M. (2014). Berries: anti-inflammatory effects in humans. *Journal of agricultural and food chemistry*, *62*(18), 3886–3903. doi.org/10.1021/jf4044056

Juranić, Z., & Zizak, Z. (2005). Biological activities of berries: from antioxidant capacity to anti-cancer effects. *BioFactors (Oxford, England)*, *23*(4), 207–211. doi.org/10.1002/biof.5520230405

Jurikova, T., Mlcek, J., Skrovankova, S., Balla, S., Sochor, J., Baron, M., & Sumczynski, D. (2016). Black Crowberry (Empetrum nigrum L.) Flavonoids and Their Health Promoting

Activity. *Molecules (Basel, Switzerland)*, *21*(12), 1685. doi.org/10.3390/molecules21121685

Jurikova, T., Sochor, J., Rop, O., Mlček, J., Balla, S., Szekeres, L., Zitný, R., Zitka, O., Adam, V., & Kizek, R. (2012). Evaluation of polyphenolic profile and nutritional value of non-traditional fruit species in the Czech Republic--a comparative study. *Molecules (Basel, Switzerland)*, *17*(8), 8968–8981. doi.org/10.3390/molecules17088968

Kähkönen, M. P., Heinämäki, J., Ollilainen, V., & Heinonen, M. (2003). Berry anthocyanins: Isolation, identification and antioxidant activities. *Journal of the Science of Food and Agriculture, 83(14),* 1403-1411. doi:10.1002/jsfa.1511.

Kalt W, Lawand C, Ryan DAJ, McDonald JE and Forney CF (2003). Oxygen radical absorbing capacity, anthocyanin and phenolic content of highbush blueberries (Vaccinium corymbosum L.) during ripening and storage. *Journal of the American Society for Horticultural Science, 128(6),* 917–23. doi: 10.21273/JASHS.128.6.0917

Kalt, W., Blumberg, J. B., McDonald, J. E., Vinqvist-Tymchuk, M. R., Fillmore, S. A., Graf, B. A., O'Leary, J. M., & Milbury, P. E. (2008). Identification of anthocyanins in the liver, eye, and brain of blueberry-fed pigs. *Journal of agricultural and food chemistry*, *56*(3), 705–712. doi.org/10.1021/jf0719981

Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & nutrition research*, *61*(1), 1361779. https://doi.org/10.1080/16546628.2017.1361779

Kim, K. A., Gu, W., Lee, I. A., Joh, E. H., & Kim, D. H. (2012). High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. *PloS one*, *7*(10), e47713. doi.org/10.1371/journal.pone.0047713

Kim, Y. S., Young, M. R., Bobe, G., Colburn, N. H & Milner, J. A. (2009). Bioactive food components, inflammatory targets, and cancer prevention. *Cancer Prevention Research, 2(3)*, 200–208. doi: 10.1158/1940-6207.CAPR-08-0141.

Kontiokari, T., Sundqvist, K., Nuutinen, M., Pokka, T., Koskela, M., & Uhari, M. (2001). Randomised trial of cranberry-lingonberry juice and Lactobacillus GG drink for the prevention of urinary tract infections in women. *BMJ (Clinical research ed.)*, *322*(7302), 1571. doi.org/10.1136/bmj.322.7302.1571

Koruk, M., Taysi, S., Savas, M. C., Yilmaz, O., Akcay, F & Karakok, M. (2004). *Annals of Clinical and Laboratory Science, 34(1),* 57–62.

Kosaka, K., Oyanagi, S., Matsushita, M., Hori, A., & Iwase, S. (1976). Presenile dementia with Alzheimer-, Pick-and Lewy-body changes. *Acta neuropathologica, 36(3),* 221-233. doi: 10.1007/BF00685366

Kowalenko, C.G. (2005). Accumulation and distribution of micronutrients in Willamette red raspberry plants. *Canadian Journal of Plant Science, 85(1),* 179–191. doi: 10.4141/P03-107.

Kowalska, K., Olejnik, A., Zielińska-Wasielica, J., & Olkowicz, M. (2019). Inhibitory effects of lingonberry (*Vaccinium vitis-idaea L.*) fruit extract on obesity-induced inflammation in 3T3-L1 adipocytes and RAW 264.7 macrophages. *Journal of Functional Foods, 54,* 371-380. doi: 10.1016/j.jff.2019.01.040

Krikorian, R., Shidler, M. D., Nash, T. A., Kalt, W., Vinqvist-Tymchuk, M. R., Shukitt-Hale, B., & Joseph, J. A. (2010). Blueberry supplementation improves memory in older adults. *Journal of agricultural and food chemistry*, *58*(7), 3996–4000. doi.org/10.1021/jf9029332

Krinsky N. I. (1992). Mechanism of action of biological antioxidants. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.)*, *200*(2), 248–254. doi.org/10.3181/00379727-200-43429

Kristián, T., & Siesjö, B. K. (1998). Calcium in ischemic cell death. *Stroke, 29(3),* 705-718, doi: 10.1161/01.STR.29.3.705

Kristo, A. S., Klimis-Zacas, D., & Sikalidis, A. K. (2016). Protective Role of Dietary Berries in Cancer. *Antioxidants (Basel, Switzerland)*, *5*(4), 37. doi.org/10.3390/antiox5040037

Kylli, P., Nohynek, L., Puupponen-Pimiä, R., Westerlund-Wikström, B., Leppänen, T., Welling, J., Moilanen, E & Heinonen, M. (2011). Lingonberry (*Vaccinium vitis-idaea*) and European Cranberry (*Vaccinium microc*arpon) Proanthocyanidins: Isolation, Identification, and Bioactivities. *Journal of Agricultural Food Chemistry, 59(7),* 3373-3384. doi: 10.1021/jf104621e.

Lagouge, M., Argmann, C., Gerhart-Hines, Z., Meziane, H., Lerin, C., Daussin, F., Messadeq, N., Milne, J., Lambert, P., Elliott, P., Geny, B., Laakso, M., Puigserver, P & Auwerx J. (2006). Resveratrol Improves Mitochondrial Function and Protects Against Metabolic Disease by Activating SIRT1 and PGC-1alpha. *Cell, 127(6),*1109-1122. doi: 10.1016/j.cell.2006.11.013.

Lätti, A. K., Riihinen, K. R., & Jaakola, L. (2011). Phenolic compounds in berries and flowers of a natural hybrid between bilberry and lingonberry (Vaccinium×intermedium Ruthe). *Phytochemistry*, *72*(8), 810–815. doi.org/10.1016/j.phytochem.2011.02.015

Leduc, C., Coonishish, J., Haddad, P., & Cuerrier, A. (2006). Plants used by the Cree Nation of Eeyou Istchee (Quebec, Canada) for the treatment of diabetes: A novel approach in quantitative ethnobotany. *Journal of ethnopharmacology*, *105*(1-2), 55–63. doi.org/10.1016/j.jep.2005.09.038

Lee, H. C., Jenner, A. M., Low, C. S., & Lee, Y. K. (2006). Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Research in microbiology*, *157*(9), 876–884. doi.org/10.1016/j.resmic.2006.07.004

Lee, J., & Finn, C. E. (2012). Lingonberry (Vaccinium vitis-idaea L.) grown in the Pacific Northwest of North America: Anthocyanin and free amino acid composition. *Journal of functional food, 4(1),* 213–218. doi.org/10.1016/j.jff.2011.10.007

Lees, A. J., Hardy, J., & Revesz, T. (2009). Parkinson's disease. *Lancet (London, England)*, *373*(9680), 2055–2066. doi.org/10.1016/S0140-6736(09)60492-X

Leighton A. L. (1985). Wild Plant Use by the Woods Cree (Nihithawak) of East-Central Saskatchewan, University of Ottawa Press.

Levine, M., Rumsey, S. C., Daruwala, R., Park, J. B., & Wang, Y. (1999). Criteria and recommendations for vitamin C intake. *JAMA*, *281*(15), 1415–1423. doi.org/10.1001/jama.281.15.1415

Lewandowsky, M. (1912). *Handbuch der neurologie*, Bd I–V.Verlag Julius Springer, Berlin.

Lipton S. A. (2008). NMDA receptor activity regulates transcription of antioxidant pathways. *Nature Neuroscience, 11(4),* 381-382. doi: 10.1038/nn0408-381.

Liu R. H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The Journal of nutrition*, *134*(12 Suppl), 3479S–3485S. doi.org/10.1093/jn/134.12.3479S

Livrea, M. A., Tesoriere, L., Bongiorno, A., Pintaudi, A. M., Ciaccio, M., & Riccio, A. (1995). Contribution of vitamin A to the oxidation resistance of human low-density lipoproteins. *Free radical biology & medicine*, *18*(3), 401–409. doi.org/10.1016/0891-5849(94)00151-9

Lobo, V., Patil, A., Phatak, A & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews, 4(8),* 118–126. doi: 10.4103/0973-7847.70902

Määttä-Riihinen, K. R., Kähkönen, M. P., Törrönen, A. R., & Heinonen, I. M. (2005). Catechins and procyanidins in berries of vaccinium species and their antioxidant activity. *Journal of agricultural and food chemistry*, *53*(22), 8485–8491. doi.org/10.1021/jf050408l

Madduma Hewage, S., Prashar, S., Debnath, S. C., O, K., & Siow, Y. L. (2020). Inhibition of Inflammatory Cytokine Expression Prevents High-Fat Diet-Induced Kidney Injury: Role of Lingonberry Supplementation. *Frontiers in medicine*, *7*, 80. doi.org/10.3389/fmed.2020.00080

Manach, C. Morand, C., Texier, O., Favier, M. L., Agullo, G., Demigné, C., Régérat, F & Rémésy, C. (1995). Quercetin Metabolites in Plasma of Rats Fed Diets Containing Rutin or Quercetin. *The Journal of Nutrition, 125(7),* 1911-22, doi: 10.1093/jn/125.7.1911.

Manach, C., Scalbert, A., Morand, C., Rémésy, C & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition, 79(5*), 727–747, doi: 10.1093/ajcn/79.5.727

Mane, C., Loonis, M., Juhel, C., Dufour, C., & Malien-Aubert, C. (2011). Food grade lingonberry extract: polyphenolic composition and in vivo protective effect against oxidative stress. *Journal of agricultural and food chemistry*, *59*(7), 3330–3339. doi.org/10.1021/jf103965b

Manganaris, G. A., Goulas, V., Vicente, A. R., & Terry, L. A. (2014). Berry antioxidants: small fruits providing large benefits. *Journal of the science of food and agriculture*, *94*(5), 825–833. doi.org/10.1002/jsfa.6432

Marambaud, P., Zhao, H., & Davies, P. (2005). Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides. *The Journal of biological chemistry*, *280*(45), 37377–37382. doi.org/10.1074/jbc.M508246200

Matsufuji, M., Otsuki, T., Takeda, T., Chino, M., & Takeda, M. (2003). Identification of reaction products of acylated anthocyanins from red radish with peroxyl radicals. *Journal of Agricultural Food Chemistry, 51(10).* 3157–3161. doi: 10.1021/jf0210122

McCallum, J. L., Yang, R., Young, J. C., Strommer, J. N., & Tsao, R. (2007). Improved high performance liquid chromatographic separation of anthocyanin compounds from grapes using a novel mixed-mode ion-exchange reversed-phase column. *Journal of chromatography. A*, *1148*(1), 38–45. doi.org/10.1016/j.chroma.2007.02.088

McConkey, D. J., & Orrenius, S. (1996). The role of calcium in the regulation of apoptosis. *Journal of Leukocyte Biology, 59(6),* 775-783. doi: 10.1002/jlb.59.6.775

McCullough, M. L., Peterson, J. J., Patel, R., Jacques, P. F., Shah, R., & Dwyer, J. T. (2012). Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults. *The American journal of clinical nutrition*, *95*(2), 454–464. doi.org/10.3945/ajcn.111.016634

Mehta, A., Prabhakar, M., Kumar, P., Deshmukh, R., & Sharma, P. L. (2013). Excitotoxicity: bridge to various triggers in neurodegenerative disorders. *European journal of pharmacology*, *698*(1-3), 6–18. doi.org/10.1016/j.ejphar.2012.10.032

Milbury, P. E., & Kalt, W. (2010). Xenobiotic metabolism and berry flavonoid transport across the blood-brain barrier. *Journal of agricultural and food chemistry*, *58*(7), 3950–3956. doi.org/10.1021/jf903529m

Milner, J. A. (1994). Reducing the risk of cancer. In *Functional foods* (pp. 39-70). Springer, Boston, MA.

Moerman, D. (1998). Native American Ethnobotany, Timber Press. Portland, Oregon.

Monaghan, B. R & Schmitt, F. O. (1932). The effects of carotene and of vitamin A on the oxidation of linoleic acid. *Journal of Biological Chemistry,*96, 387-395. doi.org/10.1016/S0021-9258(18)76278-9

Morlière, P., Patterson, L. K., Santos, C. M. M., Silva, A. M. S., Mazière, J., Filipe, P., Gomes, A., Fernandes, E., Garciah, M. B. Q & Santusc, R. (2012). The dependence of α-tocopheroxyl radical reduction by hydroxy-2,3-diarylxanthones on structure and micro-environment. *Organic and Biomolecular Chemistry, 2012(10),* 2068–2076. doi.org/10.1039/C2OB06612B

Morrell, C. N. (2008). Reactive Oxygen Species Finding the Right Balance. *Circulation Research,103(6),* 571–572. doi.org/10.1161/CIRCRESAHA.108.184325.

Morton, S., Gupta, S., & Mocibob, T., (2018), Wild blueberry powder directly from Newfoundland Canada, The Mortec Scientific Group. retrieved from, https://frozenintimeltd.com/wp-content/uploads/2018/10/Newfoundland-Blueberries-Iron-Berry-2018-White-paper.pdf

Moyer, R.A., Hummer, K.E., Finn, C.E., Frei, B., & Wrolstad, R.E. (2002). Anthocyanins, phenolics and antioxidant capacity in diverse small fruits: Vaccinium, Rubus and Ribes. *Journal of Agriculture and Food Chemistry, 50(3),* 519-525. doi: 10.1021/jf011062r

Mutanen, M., Pajari, A. M., Paivarinta, E., Misikangas, M., Rajakangas, J., Marttinen, M & Oikarinen, S. (2008). Berries as chemopreventive dietary constituents- a mechanistic approach with the $Apc^{Min/+}$ mouse. *Asia Pacific Journal of Clinical Nutrition, 17(1),*123–125.

Namiesnik, J., Vearasilp, K., Nemirovski, A., Leontowicz, H., Leontowicz, M., Pasko, P., Martinez, Ayala A.L., González-Aguilar, G.A., Suhaj M & Gorinstein, S. (2014). In vitro studies on the relationship between the antioxidant activities of some berry extracts and their binding properties to serum albumin. *Applied Biochemistry and Biotechnology, 172(6),* 2849–2865. doi: 10.1007/s12010-013-0712-2.

Nathan, C & Ding, A. (2010). Nonresolving inflammation. Cell, 140(6),871–882. doi: 10.1016/j.cell.2010.02.029.

Naumann, W. D. (1993). "Overview of the Vaccinium Industry in Western Europe". In K. A. Clayton-Greene (ed.). Fifth International Symposium on Vaccinium Culture. Wageningen, the Netherlands: International Society for Horticultural Science. pp. 53–58. ISBN 978-90-6605-475-2. OCLC 29663461.

Nifli, A. P., Kampa, M., Alexaki, V. I., Notas, G., & Castanas, E. (2005). Polyphenol interaction with the T47D human breast cancer cell line. *The Journal of dairy research*, *72 Spec No*, 44–50. doi.org/10.1017/s0022029905001172

Nijveldt, R. J., van Nood, E., van Hoorn, D. E., Boelens, P. G., van Norren, K., & van Leeuwen, P. A. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *The American journal of clinical nutrition*, *74*(4), 418–425. doi.org/10.1093/ajcn/74.4.418

Niki E. (1991). Action of ascorbic acid as a scavenger of active and stable oxygen radicals. *The American journal of clinical nutrition*, *54*(6 Suppl), 1119S–1124S. doi.org/10.1093/ajcn/54.6.1119s

Nile, S. H., & Park, S. W. (2014). Edible berries: bioactive components and their effect on human health. *Nutrition (Burbank, Los Angeles County, Calif.)*, *30*(2), 134–144. doi.org/10.1016/j.nut.2013.04.007

Ogawa, K., Kuse, Y., Tsuruma, K., Kobayashi, S., Shimazawa, M., & Hara, H. (2014). Protective effects of bilberry and lingonberry extracts against blue light-emitting diode light-induced retinal photoreceptor cell damage in vitro. *BMC complementary and alternative medicine*, *14*, 120. doi.org/10.1186/1472-6882-14-120

Ogawa, K., Sakakibara, H., Iwata, R., Ishii, T., Sato, T., Goda, T., Shimoi, K., & Kumazawa, S. (2008). Anthocyanin composition and antioxidant activity of the Crowberry (Empetrum nigrum) and other berries. *Journal of agricultural and food chemistry*, *56*(12), 4457–4462. doi.org/10.1021/jf800406v

Oh, C., Li, M., Kim, E., Park, J. S., Lee J & Ham, S. W. (2010). Antioxidant and radical scavenging activities of ascorbic acid derivatives conjugated with organogermanium. *Bulletin of the Korean Chemical Soitey, 31(12),* 3513–3514. doi:10.5012/BKCS.2010.31.12.3513

Okazaki, H., Lipkin, L. E., & Aronson, S. M. (1961). Diffuse intracytoplasmic ganglionic inclusions (Lewy type) associated with progressive dementia and quadriparesis in flexion. *Journal of Neuropathology & Experimental Neurology, 20(2),* 237-244, doi: 10.1097/00005072-196104000-00007.

Okuda, T., & Ito, H. (2011). Tannins of Constant Structure in Medicinal and Food Plants—Hydrolyzable Tannins and Polyphenols Related to Tannins. *Molecules*, *16*(3), 2191–2217. doi.org/10.3390/molecules16032191

Oldemeyer, J. L. & Seemel, R. K. (1976). Occurrence and nutritive quality of lowbush cranberry on the Kenai Peninsula, Alaska. *Canadian Journal of Botany, 54,* 966-970.

Ono, K., Yoshiike, Y., Takashima, A., Hasegawa, K., Naiki, H., & Yamada, M. (2003). Potent anti-amyloidogenic and fibril-destabilizing effects of polyphenols in vitro: implications for the prevention and therapeutics of Alzheimer's disease. *Journal of neurochemistry*, *87*(1), 172–181. doi.org/10.1046/j.1471-4159.2003.01976.x

Onwuka B and Mang B. (2018). Effects of soil temperature on some soil properties and plant growth. *Adv Plants Agric Res 8(1),* 34-37. doi: 10.15406/apar.2018.08.00288

Opal Consulting Inc. & P & G Management Services. (2010). Labrador Wild Berry Strategic Development Plan. A Strategy to Expand the Capacity of the Wild Berry Industry in Southern Labrador. Retrived from http://nakn.ca/data/4/wildberry_study-zone 4 and 5.

Padmanabhan, P., Correa-Betanzo, J., & Paliyath, G. (2016). Berries and Related Fruits. *Encyclopedia of food and health.* 364-371. doi.org/10.1016/B978-0-12-384947-2.00060-X

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of nutritional science*, *5*, e47. doi.org/10.1017/jns.2016.41

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity*, *2*(5), 270–278. doi.org/10.4161/oxim.2.5.9498

Paredes-López, O., Cervantes-Ceja, M. L., Vigna-Pérez, M., & Hernández-Pérez, T. (2010). Berries: improving human health and healthy aging and promoting quality life--a review. *Plant foods for human nutrition (Dordrecht, Netherlands)*, *65*(3), 299–308. doi.org/10.1007/s11130-010-0177-1

Park, S.Y., Lee, E.S., Han, S. H., Lee, H.Y., & Lee, S. (2011). Antioxidative effects of two native berry species, *Empetrum nigrum* var. japonicum k. Koch and *Rubus buergeri* miq, from the Jeju Island of Korea. *Journal of Food Biochem, 36.* 675–682. doi: 10.1111/j.1745-4514.201.00582.x

Parker R. S. (1996). Absorption, metabolism, and transport of carotenoids. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, *10*(5), 542–551.

Passamonti, S., Vrhovsek, U., Vanzo, A., & Mattivi, F. (2005). Fast access of some grape pigments to the brain. *Journal of agricultural and food* 7029–7034. doi.org/10.1021/jf050565k

Peng, H. W., Cheng, F. C., Huang, Y. T., Chen, C. F., & Tsai, T. H. (1998). Determination of naringenin and its glucuronide conjugate in rat plasma and brain tissue by high-performance liquid chromatography. *Journal of chromatography. B, Biomedical sciences and applications*, *714*(2), 369–374. doi.org/10.1016/s0378-4347(98)00204-7

Penhallegon, R. (2016). Lingonberry Production Guide for the Pacific Northwest. Extension service of Oregon State University, The US Department of Agriculture & Oregon Countries.

Penney, B. G., McRae, K. B., & Hall, I.V. (1985). Effect of harvest date and location on the yield of *Vaccinium vitis-idaea L.* var. minus Iodd. in Eastern Newfoundland. *Crop Research. 25(1),* 2521–2526.

Pierre, J. F., Heneghan, A. F., Feliciano, R. P., Shanmuganayagam, D., Roenneburg, D. A., Krueger, C. G., Reed, J. D., & Kudsk, K. A. (2013). Cranberry proanthocyanidins improve the gut mucous layer morphology and function in mice receiving elemental enteral nutrition. *JPEN. Journal of parenteral and enteral nutrition*, *37*(3), 401–409. doi.org/10.1177/0148607112463076

Piskula, M. K., Yamakoshi, J., & Iwai, Y. (1999). Daidzein and genistein but not their glucosides are absorbed from the rat stomach. *FEBS letters*, *447*(2-3), 287–291. doi.org/10.1016/s0014-5793(99)00307-5

Popa-Wagner, A., Mitran, S., Sivanesan, S., Chang, E., & Buga, A. M. (2013). ROS and brain diseases: the good, the bad, and the ugly. *Oxidative medicine and cellular longevity*, *2013*, 963520. doi.org/10.1155/2013/963520

Poulose, S. M., Bielinski, D. F., Carrihill-Knoll, K. L., Rabin, B. M., & Shukitt-Hale, B. (2014). Protective effects of blueberry- and strawberry diets on neuronal stress following exposure to (56) Fe particles. *Brain research*, *1593*, 9–18. doi.org/10.1016/j.brainres.2014.10.028

Prior, R. L., & Wu, X. (2006). Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. *Free radical research*, *40*(10), 1014–1028. doi.org/10.1080/10715760600758522

Rahn D. D. (2008). Urinary tract infections: contemporary management. *Urologic nursing*, *28*(5), 333–342.

Rebecca, E. Stein-Chisholm., Beaulieu, J. C., Grimm, C. C & Lloyd, S. W. (2017). LC–MS/MS and UPLC–UV Evaluation of Anthocyanins and Anthocyanidins during Rabbiteye Blueberry Juice Processing. *Beverages*, *3* (4), 56. doi.org/10.3390/beverages3040056

Reich, L. (2004). Uncommon Fruits for Every Garden. Timber press.

Reid G. (2000). Probiotic Therapy and Functional Foods for Prevention of Urinary Tract Infections: State of the Art and Science. *Current infectious disease reports*, *2*(6), 518–522. doi.org/10.1007/s11908-000-0055-3

Retsky, K. L., Freeman, M. W., & Frei, B. (1993). Ascorbic acid oxidation product(s) protect human low density lipoprotein against atherogenic modification. Anti- rather than prooxidant activity of vitamin C in the presence of transition metal ions. *The Journal of biological chemistry*, *268*(2), 1304–1309.

Reuter, S., Prasad, S., Phromnoi, K., Ravindran, J., Sung, B., Yadav, V. R., Kannappan, R., Chaturvedi, M. M., & Aggarwal, B. B. (2010). Thiocolchicoside exhibits anticancer effects through downregulation of NF-κB pathway and its regulated gene products linked to inflammation and cancer. *Cancer prevention research (Philadelphia, Pa.)*, *3*(11), 1462–1472. doi.org/10.1158/1940-6207.CAPR-10-0037

Rice-Evans, C. A., & Diplock, A. T. (1993). Current status of antioxidant therapy. *Free radical biology & medicine*, *15*(1), 77–96. doi.org/10.1016/0891-5849(93)90127-g

Riedel, G., Platt, B., & Micheau, J. (2003). Glutamate receptor function in learning and memory. *Behavioural brain research*, *140*(1-2), 1–47. doi.org/10.1016/s0166-4328(02)00272-3

Roopchand, D. E., Carmody, R. N., Kuhn. P., Moskal, K., Rojas-Silva, P & Turnbaugh, P. J. (2015). Dietary polyphenols promote growth of the gut bacterium Akkermansia muciniphila and attenuate high fat diet-induced metabolic syndromem. *Diabetes. 64(8)*, 2847–2858. doi: 10.2337/db14-1916.

Rowland, L J., & Hammerschlag, F., A. (2005). *Vaccinium spp. Blueberry.* CAB International. doi: 10.1079/9780851996622.0222.

Russo, A., Acquaviva, R., Campisi, A., Sorrenti, V., Di Giacomo, C., Virgata, G., Barcellona, M. L., & Vanella, A. (2000). Bioflavonoids as antiradicals, antioxidants and DNA cleavage protectors. *Cell biology and toxicology*, *16*(2), 91–98. doi.org/10.1023/a:1007685909018

Samad, N. B., Debnath, T., Ye, M., Hasnat, M.A & Lim, B. Q. (2014). In vitro antioxidant and anti-inflammatory activities of Korean blueberry (Vaccinium corymbosum L.) extracts. *Asian Pasific Journal of Tropical Biomedicine, 4(10),* 807–815. doi: 10.12980/APJTB.4.2014C1008.

Santangelo, C., Varì, R., Scazzocchio, B., Di Benedetto, R., Filesi, C., & Masella, R. (2007). Polyphenols, intracellular signalling and inflammation. *Annali dell'Istituto superiore di sanita*, *43*(4), 394–405.

Santos-Buelga, C & Scalbert, A. (2000). Proanthocyanidins and tannin like compounds-Nature, occurrence, dietary intake and effects on nutrition and health. *Journal of the Science of Food and Agriculture, 80(7),* 1094-1117. doi: 10.1002/(SICI)1097-0010(20000515)

Sari, E. k., Kartimo, H., Mattila, S & Tolonen A. (2006). Characterization of Phenolic Compounds from Lingonberry (Vaccinium vitis-idaea), *Journal of Agricultural Food Chemistry, 54 (26),* 9834 9842; doi: 10.1021/jf0623687.

Savaskan, E., Olivieri, G., Meier, F., Seifritz, E., Wirz-Justice, A., & Müller-Spahn, F. (2003). Red wine ingredient resveratrol protects from beta-amyloid neurotoxicity. *Gerontology*, *49*(6), 380–383. doi.org/10.1159/000073766

Scalbert, A. (1991). Antimicrobial properties of tannins. *Phytochemistry. 30(12),* 3875–3883. doi: 10.1016/0031-9422(91)83426-L

Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *The Journal of nutrition*, *130*(8S Suppl), 2073S–85S. doi.org/10.1093/jn/130.8.2073S

Schaffer, S., & Halliwell, B. (2012). Do polyphenols enter the brain and does it matter? Some theoretical and practical considerations. *Genes & nutrition*, *7*(2), 99–109. doi.org/10.1007/s12263-011-0255-5

Schofield, J., J. (1996). *Discovering Wild Plants.* Graphic Arts Center Publishing

Scott, P. J. (2010). *Edible Plants of Newfoundland and Labrador*. Boulder Publications. Portugal Cove-St. Philip's, NL.

Seeram, N. P. (2008), Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. *Journal of Agricultural Food Chemistry. 56(3),* 627-629. doi: 10.1021/jf071988k

Seeram, N. P. (2014). Berries and human health: research highlights from the Fifth Biennial Berry Health Benefits Symposium. *Journal of Agricultural Food Chemistry, 62(18):*3839–3841, doi: 10.1021/jf404349f

Sen, C. K., & Bagchi, D. (2001). Regulation of inducible adhesion molecule expression in human endothelial cells by grape seed proanthocyanidin extract. *Molecular and cellular biochemistry*, *216*(1-2), 1–7. doi.org/10.1023/a:1011053300727

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. E., Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., Setzer, W. N., … Sharifi-Rad, J. (2020). Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Frontiers in physiology*, *11*, 694. doi.org/10.3389/fphys.2020.00694

Sharma R & Thakur A. (2018). Health Promoting Phytochemicals in Vegetables: A Mini Review. *International journal of food and fermentation technology,* 8(2), 107-117. doi.org/10.30954/2277-9396.02.2018.1.

Shen-hong, X., & Hu, H. (2000). *Effects solvents and pH on DPPH assay. Journal of Instrumental Analysis.* Retrived from: http://en.cnki.com.cn/Article_en/CJFDTOTAL-TEST200003014.htm

Shi, H. L., Noguchi, N & Niki, N. (1999). Comparative study on dynamics of antioxidative action of α- tocopheryl hydroquinone, ubiquinol and α- tocopherol, against lipid peroxidation. *Free Radical Biology and Medicine, 27(3-4),* 334–46. doi: 10.1016/s0891-5849(99)00053-2.

Shukitt-Hale, B., Carey, A. N., Jenkins, D., Rabin, B. M., & Joseph, J. A. (2007). Beneficial effects of fruit extracts on neuronal function and behavior in a rodent model of accelerated aging. *Neurobiology of aging*, *28*(8), 1187–1194. doi.org/10.1016/j.neurobiolaging.2006.05.031

Shukitt-Hale, B., Thangthaeng, N., Miller, M. G., Poulose, S. M., Carey, A. N., & Fisher, D. R. (2019). Blueberries Improve Neuroinflammation and Cognition differentially Depending

on Individual Cognitive baseline Status. *The journals of gerontology. Series A, Biological sciences and medical sciences*, *74*(7), 977–983. doi.org/10.1093/gerona/glz048

Singh, A. P., & Kumar, S. (2019). Applications of Tannins in Industry. In (Ed.), Tannins - Structural Properties, Biological Properties and Current Knowledge. IntechOpen. doi.org/10.5772/intechopen.85984

Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T & Sochor, J. (2015). Bioactive Compounds and Antioxidant Activity in Different Types of Berries. *International Journal of Molecular Science, 16(10),* 24673–24706, doi: 10.3390/ijms161024673

Slatnar, A., Jakopic, J., Stampar, F., Veberic, R., & Jamnik, P. (2012). The effect of bioactive compounds on in vitro and in vivo antioxidant activity of different berry juices. *PloS one*, *7*(10), e47880. doi.org/10.1371/journal.pone.0047880

Slemmer, J. E., Shacka, J. J., Sweeney, M. I., & Weber, J. T. (2008). Antioxidants and free radical scavengers for the treatment of stroke, traumatic brain injury and aging. *Current Medicinal Chemistry, 15(4),* 404-414. doi: 10.2174/092986708783497337

Spandou, E., Soubasi, V., Papoutsopoulou, S., Augoustides-Savvopoulou, P., Loizidis, T., Pazaiti, A., Karkavelas, G., & Guiba-Tziampiri, O. (2007). Neuroprotective effect of long-term MgSO4 administration after cerebral hypoxia-ischemia in newborn rats is related to the severity of brain damage. *Reproductive Sciences, 14(7),* 667-677. doi: 10.1177/1933719107305864.

Spencer, J. P., Chaudry, F., Pannala, A. S., Srai, S. K., Debnam, E & Rice-Evans, C. (2000). Decomposition of Cocoa Procyanidins in the Gastric Milieu. *Biochemical and Biophysical Research Communication, 272(1),* 236-241. doi: 10.1006/bbrc.2000.2749.

Spillantini, M. G., Schmidt, M. L., Lee, V. M. Y., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). α-Synuclein in Lewy bodies. *Nature, 388(6645),* 839-840. doi: 10.1038/42166.

Stang, E. J., Weis, G. G & Klueh, J. (1990). *Lingonberry: Potential new fruit for the northern United States*. In: J. Janick and J.E. Simon (eds.), Advances in new crops. 321-323. Timber Press, Portland, OR.

Stefani, M., & Dobson, C. M. (2003). Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. *Journal of Molecular Medicine, 81(11),* 678-699. doi: 10.1007/s00109-003-0464-5.

Steinmetz, K. A., & Potter, J. D. (1991). Vegetables, fruit, and cancer. I. Epidemiology. *Cancer causes & control: CCC*, *2*(5), 325–357. doi.org/10.1007/BF00051672

Stewart, B.W & Wild, C.P. (2014). *World Cancer Report. World Health Organization*. Geneva, Switzerland: IARC Publication. Lyon, France.

Stintzing, F. C., Stintzing, A. S., Carle, R., Frei, B., & Wrolstad, R. E. (2002). Color and antioxidant properties of cyanidin-based anthocyanin pigments. *Journal of agricultural and food chemistry*, *50*(21), 6172–6181. doi.org/10.1021/jf0204811

Stocker, R., Bowry, V. W., & Frei, B. (1991). Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does alpha-tocopherol. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(5), 1646–1650. doi.org/10.1073/pnas.88.5.1646

Subash, S., Essa, M. M., Al-Adawi, S., Memon, M. A., Manivasagam, T., & Akbar, M. (2014). Neuroprotective effects of berry fruits on neurodegenerative diseases. *Neural regeneration research*, *9*(16), 1557–1566. doi.org/10.4103/1673-5374.139483

Sun, L., Yu, Z., Ye, X., Zou, S., Li, H., & Yu, D. (2010). A marker of endotoxemia is associated with obesity and related metabolic disorders in apparently healthy Chinese. *Diabetes Care. 33(9),* 1925–1932. doi: 10.2337/dc10-0340.

Tafazoli, S., Wright, J. S & O'Brien, P. J. (2005). Prooxidant and antioxidant activity of vitamin E analogues and troglitazone. *Chemical Research in Toxicology, 18(10),* 1567–1574, doi: 10.1021/tx0500575.

Talavéra, S., Felgines, C., Texier, O., Besson, C., Gil-Izquierdo, A., Lamaison, J. L., & Rémésy, C. (2005). Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. *Journal of agricultural and food chemistry*, *53*(10), 3902–3908. doi.org/10.1021/jf050145v

Talavéra, S., Felgines, C., Texier, O., Besson, C., Gil-Izquierdo, A., Lamaison, J. L., & Rémésy, C. (2005). Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. *Journal of agricultural and food chemistry*, *53*(10), 3902–3908. doi.org/10.1021/jf050145v

Tanaka, T., Matsuo, Y., & Saito, Y. (2018). Solubility of tannins and preparation of oil-soluble derivatives. *Journal of oleo science*, *67*(10), 1179–1187. doi.org/10.5650/jos.ess18164

Tesoriere, L., D'arpa, D., Re, R & Livrea, M. A. (1997). Antioxidant reactions of all-trans retinol in phospholipid bilayers: effect of oxygen partial pressure, radical fluxes, and retinol concentration. *Archives of Biochemistry and Biophysics, 343(1),* 13–18, doi: 10.1006/abbi.1997.0128.

Tian, Y., Liimatainen, J., Alanne, A. L., Lindstedt, A., Liu, P., Sinkkonen, J., Kallio, H., & Yang, B. (2017). Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. *Food chemistry*, *220*, 266–281. doi.org/10.1016/j.foodchem.2016.09.145

Tirmenstein, D. (1991). *Vaccinium vitis-idaea*. In: Fire Effects Information System, USA. (Online).

Toescu, E. C. (1998). Apoptosis and cell death in neuronal cells: where does Ca2+ fit in?. *Cell Calcium, 24(5-6),* 387-403. doi: 10.1016/s0143-4160(98)90062-8.

Torabi S. (2013). Statistical overview of Canadian fruit industry. Agriculture and Agri food Canada, Retrieved from, https://publications.gc.ca/collections/collection_2015/aac-aafc/A71-33-2013-eng.pdf

Trump, B. F., & Berezesky, I. K. (1995). Calcium-mediated cell injury and cell death. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, *9*(2), 219–228. doi.org/10.1096/fasebj.9.2.7781924

Tsao, R. (2010). Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients, 2(12)*, 1231–1246, doi: 10.3390/nu2121231

Tsao, R., & McCallum, J. (2009). *Fruit and Vegetable Phytochemicals: Chemistry, Nutritional Value, and Stability.* Wiley-Blackwell. doi:10.1002/9780813809397.

Turner., Adam, F. & Szczawinski, N. J. (1979). *Edible Wild Fruits and Nuts of Canada.* National Museum of Natural Sciences.

Valenzano, D. R., & Cellerino, A. (2006). Resveratrol and the Pharmacology of Aging: A New Vertebrate Model to Validate an Old Molecule. *Cell Cycle, 5(10),* 1027-32. doi: 10.4161/cc.5.10.2739.

Vendrame, S., Del Bo', C., Ciappellano, S., Riso, P., & Klimis-Zacas, D. (2016). Berry Fruit Consumption and Metabolic Syndrome. *Antioxidants (Basel, Switzerland)*, *5*(4), 34. doi.org/10.3390/antiox5040034

Vieira, A. V., Schneider, W. J., & Vieira, P. M. (1995). Retinoids: transport, metabolism, and mechanisms of action. *The Journal of endocrinology*, *146*(2), 201–207. doi.org/10.1677/joe.0.1460201

Vincent, P., & Mulle, C. (2008). Kainate receptors in epilepsy and excitotoxicity. *Neuroscience, 158(1),* 309-323. doi: 10.1016/j.neuroscience.2008.02.066.

Visti, A., Viljakainen, S., & Laakso, S. (2003). Preparation of fermentable lingonberry juice through removal of benzoic acid by Saccharomyces cerevisiae yeast. *Food Research International, 36(6),* 597-602. doi: 10.1016/S0963-9969(03)00007-3

Vojdani, A., Bazargan, M., Vojdani, E., & Wright, J. (2000). New evidence for antioxidant properties of vitamin C. *Cancer detection and prevention*, *24*(6), 508–523.

Vrieze, A., Van, N. E., Holleman, F., Salojarvi, J., Kootte, R. S & Bartelsman, J. F. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology, 143(4),* 913–916. doi: 10.1053/j.gastro.2012.06.031.

Vyas, P., Kalidindi, S., Chibrakova, L., Igamberdiev, A. U., & Weber, J. T. (2013). Chemical Analysis and effects of blueberry and lingonberry fruits and leaves against glutamate-mediated excitotoxicity. *Journal of Agricultural Food Chemistry, 61(32),* 7769-7776. doi: 10.1021/jf401158a.

Wallace, T. C., & Giusti, M. M. (2015). Anthocyanins. *Advances in Nutrition, 6(5),* 620-622, doi: org/10.3945/an.115.009233.

Walther, B. (2018). 5 things you didn't know about wild blueberries. Retrieved from, http://www.readersdigest.ca/food/healthy-food/5-things-didnt-know-wild-blueberries/view-all/

Wang, H., Cao, G., & Prior, R. L. (1997). Oxygen radical absorbing capacity of anthocyanins. *Journal of Agricultural Food Chemistry, 45(2),* 304–309. doi.org/10.1021/jf960421t.

Wang, S. Y., & Jiao, H. J. (2000). Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *Journal of Agricultural food Chemistry, 48 (11),* 5677-5684. doi:10.1021/jf000766i

Wang, S. Y., & Lin, H. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agricultural food Chemistry, 48(2),*140-146. doi: 10.1021/jf9908345.

Wang, S. Y., & Stretch, A. W. (2001). Antioxidant capacity in cranberry is influenced by cultivar and storage temperature. *Journal of Agricultural food Chemistry,49(2),* 969-974. doi: 10.1021/jf001206m.

Wang, S. Y., Feng, R., Linda B., Penhallegon, R., Ding, M., & Lu, Y. (2005). Antioxidant Activity in Lingonberries (*Vaccinium vitis-Idaea L.*) and Its Inhibitory Effect on Activator protein-1, Nuclear factor-kappaB, and Mitogen-Activated Protein Kinases Activation. Journal of Agricultural food Chemistry. *Journal of Agricultural Food Chemistry, 53(8),* 3156-66, doi: 10.1021/jf048379m.

Weber J. T. (2012). Altered calcium signaling following traumatic brain injury. *Frontiers in pharmacology*, *3*, 60. doi.org/10.3389/fphar.2012.00060

Weber J.T., (2004). *Calcium Homeostasis Following Traumatic Neuronal Injury, Current Neurovascular Research.* Bentham science. Publishers. doi.org/10.2174/1567202043480134

Wedick, N. M., Pan, A., Cassidy, A., Rimm, E. B., Sampson, L., Rosner, B., Willett, W., Hu, F. B., Sun, Q., & van Dam, R. M. (2012). Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. *The American journal of clinical nutrition*, *95*(4), 925–933. doi.org/10.3945/ajcn.111.028894

Whyte, A. R., & Williams, C. M. (2015). Effects of a single dose of a flavonoid-rich blueberry drink on memory in 8 to 10 y old children. *Nutrition (Burbank, Los Angeles County, Calif.)*, *31*(3), 531–534. doi.org/10.1016/j.nut.2014.09.013

Whyte, A. R., Schafer, G., & Williams, C. M. (2016). Cognitive effects following acute wild blueberry supplementation in 7- to 10-year-old children. *European journal of nutrition*, *55*(6), 2151–2162. doi.org/10.1007/s00394-015-1029-4

Wigner, P., Czarny, P., Galecki, P., & Sliwinski, T. (2017). Oxidative and Nitrosative Stress as Well as the Tryptophan Catabolites Pathway in Depressive Disorders. *Psychiatria Danubina*, *29*(4), 394–400. doi.org/10.24869/psyd.2017.394

Williams, R. J., Spencer, J. P., & Rice-Evans, C. (2004). Flavonoids: antioxidants or signalling molecules?. *Free radical biology & medicine*, *36*(7), 838–849. doi.org/10.1016/j.freeradbiomed.2004.01.001

Williamson, G. (2017). The role of polyphenols in modern nutrition. *Nutritional Bulletin, 42(3),* 226–235. doi: 10.1111/nbu.12278

Williamson, G., & Clifford, M. N. (2010). Colonic metabolites of berry polyphenols: the missing link to biological activity?. *The British journal of nutrition*, *104 Suppl 3*, S48–S66. doi.org/10.1017/S0007114510003946

Witting, P. K., Upston J. M & Stocker, R. (1997). Role of α-Tocopheroxyl Radical in the Initiation of Lipid Peroxidation in Human Low-Density Lipoprotein Exposed to Horse Radish Peroxidase. *Biochemistry, 36(6),* 1251–1258. doi: 10.1021/bi962493j

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2006). Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *Journal of agricultural and food chemistry*, *54*(11), 4069–4075. doi.org/10.1021/jf060300l

Youdim, K. A., & Joseph, J. A. (2001). A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. *Free radical biology & medicine*, *30*(6), 583–594. doi.org/10.1016/s0891-5849(00)00510-4

Youdim, K. A., Dobbie, M. S., Kuhnle, G., Proteggente, A. R., Abbott, N. J., & Rice-Evans, C. (2003). Interaction between flavonoids and the blood-brain barrier: in vitro studies. *Journal of neurochemistry*, *85*(1), 180–192. doi.org/10.1046/j.1471-4159.2003.01652.x

Youdim, K. A., Qaiser, M. Z., Begley, D. J., Rice-Evans, C. A., & Abbott, N. J. (2004). Flavonoid permeability across an in situ model of the blood-brain barrier. *Free radical biology & medicine*, *36*(5), 592–604. doi.org/10.1016/j.freeradbiomed.2003.11.023

Young, I. S., & Woodside, J. V. (2001). Antioxidants in health and disease. *Journal of clinical pathology*, *54*(3), 176–186. doi.org/10.1136/jcp.54.3.176

Yu, S. Y., Wu, D. C., Liu, L., Ge, Y., & Wang, Y. T. (2008). Role of AMPA receptor trafficking in NMDA receptor-dependent synaptic plasticity in the rat lateral amygdala. *Journal of neurochemistry*, *106*(2), 889–899. doi.org/10.1111/j.1471-4159.2008.05461.x

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64(4),* 555-559. doi.org/10.1016/S0308-8146(98)00102-2.

Zhou, Y., Wang, J., Gu, Z., Wang, S., Zhu, W., Aceña, J. L., Soloshonok, V. A., Izawa, K., & Liu, H. (2016). Next Generation of Fluorine-Containing Pharmaceuticals, Compounds Currently in Phase II-III Clinical Trials of Major Pharmaceutical Companies: New Structural Trends and Therapeutic Areas. *Chemical reviews*, *116*(2), 422–518. doi.org/10.1021/acs.chemrev.5b00392.