UNIVERSITÉ DU QUÉBEC

MÉMOIRE PRÉSENTÉ À
L'UNIVERSITÉ DU QUÉBEC À TROIS-RIVIÈRES

COMME EXIGENCE PARTIELLE
DE LA MAÎTRISE EN SCIENCES DE L'ENVIRONNEMENT

PAR
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MISE AU POINT D'UN PROCÉDÉ EFFICACE POUR RÉCUPÉRER LES POLYPHÉNOLS ANTIOXYDANTS DE LA CAMARINE NOIRE (*EMPETRUM NIGRUM*) AVEC DES EXTRACTIONS ASSISTÉES PAR SOLVANT ET MICRO-ONDES

DEVELOPMENT OF AN EFFECTIVE PROCESS TO RECOVER ANTIOXIDANT ACTIVE POLYPHENOLS FROM CROWBERRIES (*EMPETRUM NIGRUM*) WITH SOLVENT AND MICROWAVE-ASSISTED EXTRACTIONS

DÉCEMBRE 2019

ACKNOWLEDGMENTS

I would like to express the deepest appreciation to my research director, Professor Simon Barnabé for the opportunity to become a pioneer and be one of the first two people to complete a master's degree in the remote region of the Basse-Côte-Nord du Québec; something that was once thought unattainable. His guidance, enthusiasm and vision for this project has provided me with a rewarding experience, one that I will not forget. I would also like to thank my co-director, AgatheVialle for her support, advice and suggestions on all my questions and research that was sent her way. I offer many thanks to Benjamin Boëns and Amadou Diop for investing their time and help in the laboratory; from locating and setting up equipment to reviewing protocols and chemistry techniques as well as Kokou Adjallé for helping with my many laboratory protocols. I also thank Maxim Tardif, Louis-Charles Rainville, Isabel Desgagné-Penix, Nathalie Bourdeau, Julien Bley and Alain Tremblay for their suggestions and helpful advice on my work.

I would like to thank all of my laboratory colleagues for giving me advice and helping me in any way throughout the two years. I would especially like to give thanks to Jessica Poole, my teammate and good friend during this project. We spent many hours together performing experiments, finding and analyzing protocols and discussing calculations and results. Together, we combined our knowledge and problem solved anything that came our way and I will not forget this experience of ours.

To my parents, John W. Roberts and Elaine Nadeau and siblings, Candace and Brittanie Roberts who have always pushed me to be the best I can be in all aspects of life; I would not have come this far without them. They have always supported me throughout all my studies and for that, I am eternally grateful.

And finally, I thank Kimberly Buffitt for taking this remarkable initiative and allowing me to be able to return to the Basse-Côte-Nord and obtain my master's degree

in my field. This has been an amazing opportunity, one that I could have only dreamed, and I am so very thankful I was able to be a part of this exciting journey and I am excited for what is to come. Lastly, I thank the Lower North Shore Bioproducts Solidarity Cooperative, the Coasters Association Inc, the consortium for research and innovation in industrial bioprocesses in Québec (CRIBIQ) and the MITACS program for their important financial assistance.

FOREWARD

This thesis is subject to work carried out from May 2016 to December 2018 as part of the master's degree in Environmental Science. The project was conducted under the supervision of Professor Simon Barnabé in the Department of Chemistry, Biochemistry and Physics. This thesis also presents the article entitled "Obtaining antioxidant active polyphenol extracts from the crowberry *Empetrum nigrum* L. on the Basse-Côte-Nord du Québec" which was submitted to the journal "Industrial Biotechnology" on February 15th 2019.

This master's degree project began with a collaboration with the Lower North Shore Bioproducts Solidarity Cooperative which is a company that are interested in beginning the process of potentially using the wildberry, *Empetrum nigrum* or crowberry, from the region in nutraceutical and cosmeceutical products. The Lower North Shore Bioproducts Solidarity Cooperative is a cooperative business that was put into place to stimulate economic diversification in the communities through sustainable exploitation of natural-based resources and also to become a world leader in exploiting and developing bio-ingredients in a sub-arctic region. It was initiated in 2006 with a small group of women who called themselves the BerryLicious Committee. The committee sought to create an enterprise by developing wildberry recipes and creating food products. Today, the Lower North Shore Bioproducts Solidarity Cooperative has built a processing factory which was completed in 2015 in the Bonne-Espérance region. The food production of wildberry-based food products began in 2016.

However, some issues surrounding this realization was the berry from the region has never been tested for phenolic or antioxidant content and therefore no published data. Also, the Basse-Côte-Nord is a remote with a number of complex issues when it comes to performing extractions such as no roads connecting to the rest of the province of Québec nor proper waste disposal for hazardous chemicals just as examples. Thus, the objective of this project was to sample and analyze crowberries from the region for phenolic content

and antioxidant activity using techniques that could be replicated on the Basse-Côte-Nord that are both suitable for the region and potential integration into natural health and cosmetic products. Although there is some research on the crowberries from Europe, the biological activities from the crowberries of the Basse-Côte-Nord are unknown. Thus, experts and researchers from the Université du Québec à Trois-Rivières and their partners have used their knowledge to aid in finding solutions for the company. So far, the Lower North Shore Bioproducts Solidarity Cooperative has begun to move forward with ingredient companies located around the world (China, United States) to develop and sell wildberry extracts from the local biomasses. In 2018, the factory produced 15,000 units of the cosmetic cream, "Ampersand", made with local wildberry extracts were mixed, filled and packaged for the cosmetic company L'Onvie. Therefore, the transition into wildberry extracts has begun, but it is essential to learn as much as possible about wildberries and their properties.

Therefore, this project, carried out by my colleague, Jessica Poole and myself, had the fundamental objective of carrying out extractions on two different species of plants from the Basse-Côte-Nord: a marine plant, *Ascophyllum nodosum* and a land plant, *Empetrum nigrum* respectively, to determine if phenolic content and antioxidant activity were present to be potentially used in nutraceuticals and cosmeceuticals. The results shown in this thesis account for the general progress on the project of potential plants of the Basse-Côte-Nord that could be used in future products.

RÉSUMÉ

Ces dernières années, l'importance des produits de santé naturels et des cosmétiques utilisant la chimie verte et les ingrédients naturels et biologiques est un sujet d'intérêt croissant car les entreprises manufacturières et la population comprennent de mieux en mieux à quel point certains produits de santé et les ingrédients cosmétiques traditionnels peuvent être nocifs. La plupart des entreprises manufacturières ont réorienté leur intérêt et leurs recherches vers le développement d'ingrédients capables de répondre à cette demande croissante. Plusieurs des ingrédients naturels faisant l'objet de recherche scientifique sont d'origine végétale, comme les fruits, les légumes, les plantes, les algues, etc. et il existe de nombreuses études publiées sur leurs activités biologiques et les avantages de ces additifs naturels dans les produits de santé et les cosmétiques. Les baies font partie des fruits actuellement utilisés dans les produits et sont de plus en plus populaires en raison de leurs propriétés antioxydantes.

Étant donné l'abondance de la camarine noire (*Empetrum nigrum*) dans la région de la Basse Côte Nord du Québec (Canada) et le fait que ces baies sont déjà utilisées dans les pays européens et connues pour leurs propriétés antioxydantes, il y a intérêt à étudier davantage la camarine pour ses composés phénoliques et son activité antioxydante en utilisant des techniques d'extraction appropriées pour une région éloignée avec des problèmes complexes liés au transport, aux équipements et au traitement des déchets. Ainsi, l'objectif du projet de maîtrise était de préparer des extraits de camarine noire et plus précisément d'analyser le contenu phénolique total ainsi que l'activité antioxydante en déterminant la teneur en anthocyanine. Pour ce faire, quatre objectifs avaient été fixés : (1) préparer et de conserver les échantillons de camarine par des techniques efficaces pour ne pas altérer les propriétés antioxydantes et par des techniques adaptées à la région comme l'utilisation d'extraits congelés pour les extraits plutôt que lyophilisés; (2) examiner deux méthodes d'extraction différentes pour déterminer le procédé le plus efficace tout en tenant compte des limites de la région; (3) déterminer si la peau des baies et les résidus de graines de la camarine ont un contenu phénolique et une activité antioxydante similaires en reproduisant les expériences faites avec les échantillons de camarine entière (cela permettrait d'utiliser 100 % de la camarine que d'avoir des peaux et des graines comme déchets provenant des extractions); (4) déterminer s'il y a suffisamment de contenu phénolique et d'activité antioxydante présents pour être potentiellement utilisés dans une formulation cosmétique en déterminant si elle est comparable aux autres fruits déjà utilisés dans les cosmétiques et les produits de santé naturels.

Deux méthodes d'extraction ont été appliqués sur les baies, soit l'extraction solide-liquide et l'extraction assistée par micro-ondes sous différents paramètres d'extraction dont la température, la concentration en solvant et le temps. Les mélanges d'éthanol dans l'eau ont généralement donné les plus hauts rendements en contenu phénolique total pour les deux méthodes d'extraction. L'activité antioxydante a aussi été analysée et les résultats indiquent que la camarine a une forte activité antioxydante, particulièrement en utilisant

des mélanges d'éthanol dans l'eau et la méthode d'extraction par micro-ondes. Cela est comparable à d'autres baies sauvages déjà utilisées dans les cosmétiques commerciaux et les produits de santé. En général, les résultats obtenus entre les deux méthodes d'extraction ont montré que la méthode d'extraction assistée par micro-ondes permettait généralement des rendements légèrement supérieurs à ceux de la méthode d'extraction solide-liquide. Cette méthode a également permis d'utiliser moins de solvant lors de l'analyse du contenu phénolique total et de réduire considérablement le temps d'extraction. Les résultats de ce projet démontrent l'intérêt d'utiliser des extraits de camarine noire comme source de composés antioxydants et phénoliques et la possibilité de faire de tels extractions dans des régions nordiques éloignées pour un plus grand impact socio-économique local.

Mots-clés : camarine noire, antioxydants, extraction assistée par micro-ondes, *Empetrum nigrum*, polyphénols

ABSTRACT

In recent years, the importance of natural health products and cosmetics using green chemistry and natural and organic ingredients is a growing movement because it is becoming more understood how damaging traditional health product and cosmetic ingredients can be. Most companies who create these products have shifted their interest and research into finding and developing ingredients that can compete with this growing demand. Furthermore, there are many published studies analyzing the biological activities and benefits of natural additives such as fruits, algae and so on. In this study, we are interested in analyzing crowberries (*Empetrum nigrum*) from the Basse-Côte-Nord du Québec to determine its phenolic content and antioxidant activity using suitable techniques for a remote region with complex issues surround transportation, equipment and proper waste disposal. To achieve this goal, the crowberries were extracted using two extraction methods: solid-liquid extraction and microwave-assisted extraction using different extraction parameters such as temperature, solvent concentrations and time. The total phenolic content was analyzed using the Folin-Ciocalteu assay and revealed the crowberry has an abundance of phenolic content where the ethanol mixtures in water generally gave the highest yields of total phenolic content for both extraction methods. Using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, the antioxidant activity was analyzed, and the results indicate that the crowberry has strong antioxidant activity especially using ethanol mixtures in water and the microwave extraction method. It is comparable to other fruits already used in commercial cosmetic and health products as well. Generally, the results between the two extraction methods determined that the microwave-assisted extraction method generally allowed for slightly higher yields than the solid-liquid extraction method. This method also allowed for the use of less solvent when analyzing for total phenolic content and significantly less extraction time all around. Therefore, the overall results of this study demonstrate the verifiable interest of using crowberry extracts as a source for antioxidant and phenolic compounds.

Keywords: crowberry, antioxidants, microwave-assisted extraction, *Empetrum nigrum*, extraction, polyphenols

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LIST OF ABBREVIATIONS AND ACRONYMS

DPPH	2,2-diphenyl-1-picrylhydrazyl
CCTT	Centres collégiaux de transfert de technologie
GAE	Gallic acid equivalents
HPLC	High performance liquid chromatography
IC50	Concentration required to scavenge 50% of DPPH reagent
LC-MS	Liquid chromatography – mass spectrometry
MAE	Microwave-assisted extraction
MAP	Monomeric anthocyanin pigments
SLE	Solid-liquid extraction
UQTR	Université du Québec à Trois-Rivières

LIST OF SYMBOLS

°C	Degrees Celsius
CH3CO2Na	Sodium acetate
cm	Centimeter
g	Gram
HCl	Hydrochloric acid
KCl	Potassium chloride
Mg	Milligram
ml	Milliliter
Mm	Millimolar
Na_2CO_3	Sodium carbonate
Nm	Nanometer
µg	Microgram
µl	Microliter
% v/v	Volume/volume percent
W	Watts

CHAPTER I

INTRODUCTION

1.1 Current context of the Basse-Côte-Nord du Québec

1.1.1 Current economic situation

The Basse-Côte-Nord du Québec is situated along three hundred and seventy five kilometers along the Gulf of St. Lawrence and has a population of about five thousand comprised of five municipalities and sixteen small, remote English, French and Innu villages which are unconnected by road to the rest of the province of Québec as seen in Figure 1. Traditionally, the entirety of the regional income is largely centralized around the fishing industry. In 2004, the communities faced the closure of the zone thirteen cod fishery as well as substantial quota reduction in the crab fishery. This devitalized many communities to a less than poor economic state and a very large unemployment rate. In the past decade, many socio-economic stakeholders and businesses have been investing knowledge to create new economic diversification initiatives to develop other sources of economy such as non-timber forest products. For example, a list of over sixty-five species of non-timber forest products have been identified to date which include both marine and land plants such as wildberries, peat moss and algae [1].

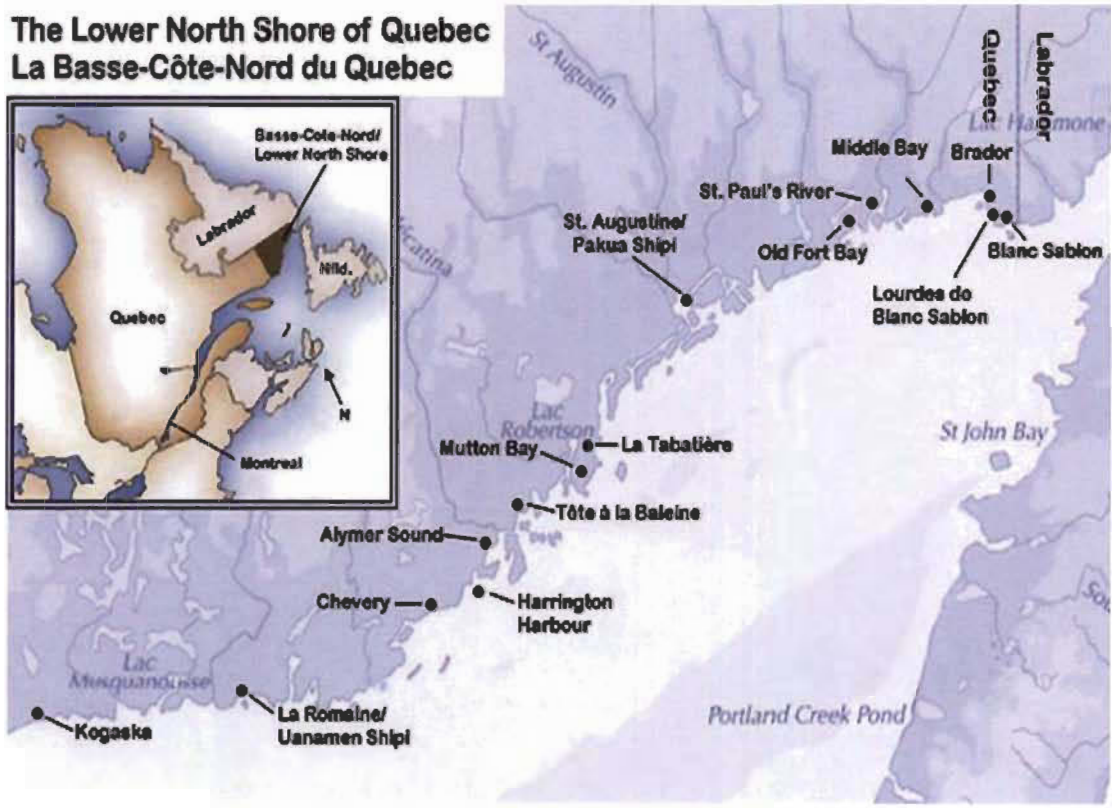


Figure 1.1 Geographical map of the communities of the Basse-Côte-Nord in relation to the province of Québec [1].

1.1.2 Current ecological situation

Empetrum nigrum L., commonly known as the crowberry and locally known on the Basse-Côte-Nord as the blackberry is identified as one of the sixty-five species of non-timber forest products that has the significant potential to be developed by the Lower North Shore Bioproducts Solidarity Cooperative. They are black-purple in color and grow in light green, mat forming shrubs which occur in northern regions, mostly in the arctic tundra biome at higher latitudes. On the Basse-Côte-Nord, the crowberry biomass grows in and around all of the communities in great abundance and are harvested every year in the late summer by the local residents. There is also over sixty-five thousand square kilometres of land that belongs to the local MRC which remains virtually untouched to this day. The crowberries are consumed fresh and are also frozen for future cooking purposes or produced into jams for the over six-month winter season.

Crowberries have a very high antioxidant level and its extracts could be utilized in natural health and cosmetic products which in turn could expand the local economy [2]. Today, most cosmetic and health companies are looking for alternative products where the constituents of the contents is derived from natural and organic means and moving away from synthesized materials. For example, The Innovation Company uses Nordic berries from Finland for their cosmetic products. Other examples include the well-known company Aveeno which recently released a blackberry-infused cream line. Thus, it can be seen that the growing demand for natural materials such as fruits are emerging to be a potential solution for cosmetic and health companies. There is a vast abundance of crowberries and one particular advantage of the crowberry biomass on the Basse-Côte-Nord, is that it is located in a pristine environment. The crowberry biomass is exempt of contaminants such as heavy metals and pesticides according to its geographical localization because it very isolated from human and industrial activities. Hence, it is essential to develop this resource for economic diversification of the region. It is also important that the development of this resource comes with sustainable harvesting practices to ensure it is not depleted.

1.2 Properties of berries

1.2.1 Phytochemicals

Phytochemicals are chemicals compounds that are produced by plants through primary or secondary metabolism with the intention of helping them thrive in an environment or thwart pathogens and competitors [3,4]. Primary plant metabolites are essential and are directly involved in the growth and development of the plant whereas secondary metabolites, which are important to the plant although not essential, are produced in the metabolic pathways [4]. Phytochemicals can be classified into major categories such as carotenoids, polyphenols which include the sub-categories of phenolic acids, flavonoids and stilbenes/lignans [5,6] as seen in Figure 1.2. The sub-group of flavonoids can be further divided into groups of a similar chemical structure which include anthocyanins, flavones, flavanones, isoflavones and flavanols [5,6]. Berries are a rich

source of phytochemicals especially the groups of polyphenols in particular the flavonoid group [7,8].

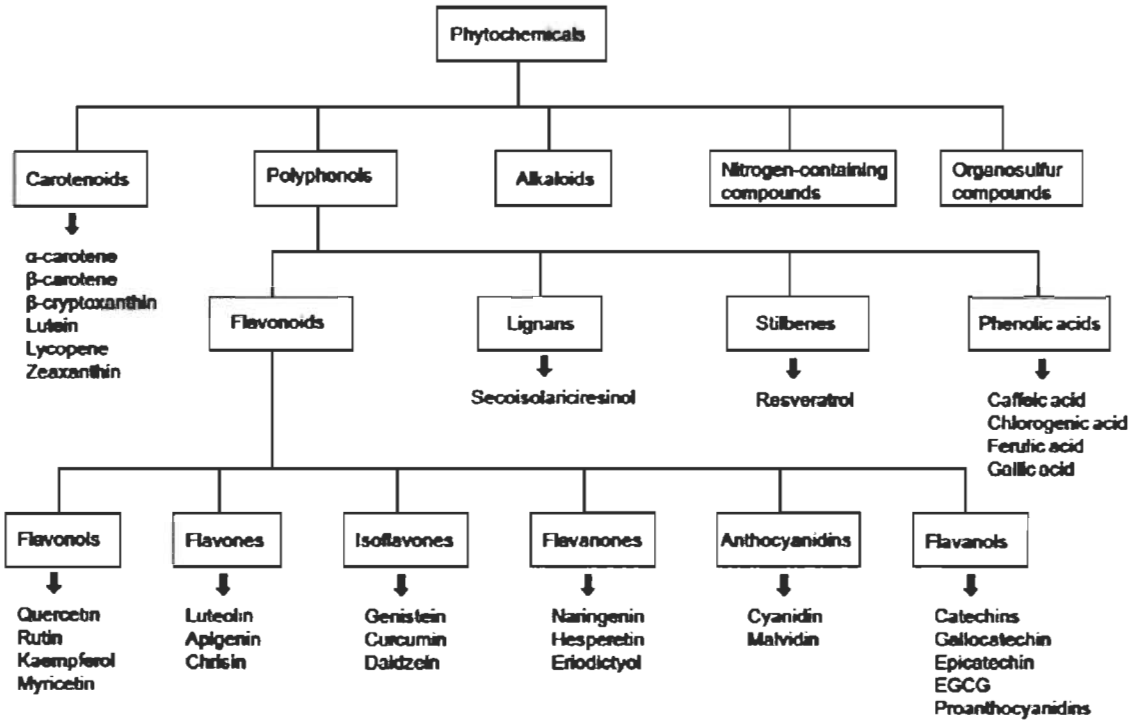


Figure 1.2 Phytochemicals and its subgroupings [11].

1.2.2 Phenolic compounds and its characteristics

Polyphenolic compounds are present in all plant organs and therefore a part of the everyday human diet. Phenolic compounds are the derivatives of pentose phosphate, shikimate, and phylpropanoid pathways in plants [3]. These compounds are one of the most widely distributed substances in the plant kingdom where there are more than 8000 phenolic structures presently known [10]. Structurally, phenolics compounds are comprised of an aromatic ring with one or more hydroxyl substituents and they can be very simple phenolic compounds such as phenolic acids or very polymerised compounds such as lignins. [3,11] as seen in Figure 1.3. They are generally classified based on the number of carbon atoms present in the molecule [12,13]. Phenolics are most often produced in the sub-epidermal layers of the plant tissues that have been exposed to a negative event such as a pathogen or predator or stress [12,14,15]. Phenolic compounds

are part of the cell wall support structure and are produced when the plant is under stressful conditions which include infections, wounds and UV exposure [16]. Considering plants produce phenolic compounds when they are under stress, could be why some research suggests there are more phenolic compounds in the skins of the berry [17]. There are several factors that affect the concentration of a particular phenol compound with the plant tissue such as traumas, wounds or pathogens [12,18-20]. It is also dependent on the season as well as the growth stage of the plant [12,18]. In some species of plants, nutrient stresses can also trigger the production of particular phenolic compounds such as lack of nitrogen, phosphate and potassium [21]. Phenolic compounds are proposed to have a preventative role in illnesses such as cancer development and heart disease; an example of this health benefit is the reasonable ingestion of alcohol-free red wine where the grapes used have a mixture of phenolic compounds which has been shown to improve the antioxidant status of plasma in humans [22-24].

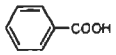
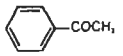
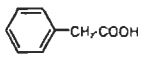
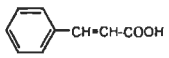
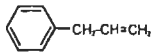
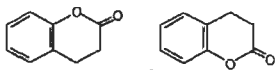
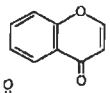
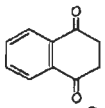
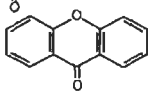
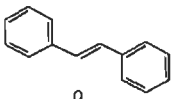
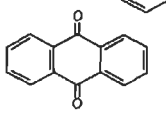
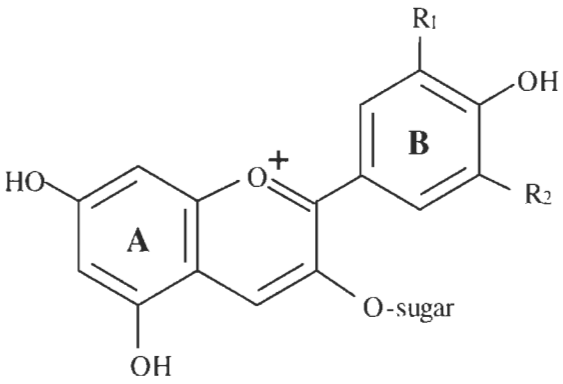
Class	Basic Skeleton	Basic Structure
Simple phenols	C_6	
Benzoquinones	C_6	
Phenolic acids	C_6-C_1	
Acetophenones	C_6-C_2	
Phenylacetic acids	C_6-C_2	
Hydroxycinnamic acids	C_6-C_3	
Phenylpropenes	C_6-C_3	
Coumarins, isocoumarins	C_6-C_3	
Chromones	C_6-C_3	
Naftoquinones	C_6-C_4	
Xanthones	C_6-C_1-C_6	
Stilbenes	C_6-C_2-C_6	
Anthraquinones	C_6-C_2-C_6	
Flavonoids	C_6-C_3-C_6	
Lignans, neolignans	$(C_6$-$C_3)_2$	
Lignins	$(C_6$-$C_3)_n$	

Figure 1.3 Main classes of polyphenolic compounds [11].

1.2.2.1 Anthocyanins and its characteristics

Anthocyanins which in Greek *Anthos* means flower, and *kyanos* means blue are glycoside-derivatives of anthocyanidins, meaning an anthocyanin is formed when a sugar moiety is attached to the functional group of the anthocyanidin structure via a glycosidic bond. There are six Anthocyanidins that are known in the plant kingdom: cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin where cyanidin is the most common in nature. They form conjugates with a variety of sugars where the glycosides of cyaidin, dephinidin and pelargondin are the most widespread in nature, being present in

80% of pigmented leaves, 69% of fruits and 50% of flowers. [26,27]. Anthocyanins have redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and in addition they have a metal chelation potential [28].



Anthocyanidin	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvinidin	OCH_3	OCH_3

Figure 1.4 Structures of anthocyanidins [25].

They are compounds that are visible to the human eye where they vary from pink to blue to violet. [27,29,30]. Anthocyanins are the largest group of water-soluble pigments in the plant kingdom and are present in almost all of the higher plants but are more noticeable in fruiting and flowering plants [31,32]. They have several essential roles in the plants including plant reproduction by attracting pollinators and seed dispersers [33]. Apart from being widely distributed in the fruit and floral tissues, they are also located in the roots, shoots and leaves. They have been reported to be found in the root cap of *Impatiens* seedlings as well as the root when they are exposed to stress, pathogens or osmotic pressure [32,34-36]. Anthocyanin compounds are also present in the stems of plants when they experience osmotic stress and cold temperatures [32,37]. In certain plant species, the anthocyanins are present in the leaves, in the sub-epidermis of the leaf. The group of polyphenols and sub-group of flavonoids and thus anthocyanins are largely present in plants and is one of the many reasons they are sought after; these compounds can have many biological and health benefits.

1.2.3 Biological activities and health benefits

In addition to their functions in plants, both phenolic compounds and anthocyanins in particular, have many other benefits. In recent years, there has been numerous studies that attest to these compounds possessing biological activities with major health benefits such as anticarcinogenic, anti-inflammatory, antihepatotoxic, antibacterial, antiviral, antiallergenic, antithrombotic, antioxidant activities and the prevention of DNA damage [28,29,38-45]. Anthocyanins show potential as free radial scavengers which could lead to a potential supplement for chronic degenerative diseases associated with oxidative stress [40,46]. In another study, they have found the fruit extracts with anthocyanins were effective in reversing age-related deficits in several neural and behavioral parameters [27,47]. An antioxidant study of anthocyanins fractions from an Italian red wine have shown that the anthocyanin fractions was most effective in free radical scavenging and therefore a key component that can protect against cardiovascular disease in red wine [27,47]. Also, numerous studies examining human consumption show increase health. There were positive effects of berries and their seeds on the cardiovascular system where there was improvement in the plasma lipid profile, endothelial function of the blood vessels and reduction of blood pressure and platelet aggregation [48-50].

Along with the phytochemicals in the berries, they also contain several important micronutrients that are beneficial to the health as well. Many types of berries contain vitamin C in so much abundance that often just one handful can provide a person with their daily intake [26]. Vitamin C plays a role in many pathways including collagen synthesis, the immune system and hormone synthesis [26,51,52]. Another micronutrients that is plentiful in berries are folic acid which plays a crucial role in preventing neural tube defects in new born babies [26]. There are countless studies attesting to the health and biological benefits of berries and now the benefits of the usually underutilized wildberries are coming to light.

1.2.4 Wildberries in the northern hemisphere

Wildberries are small, wild fruits that come in a variety of species that are found all over Canada. They can grow in many different climatic conditions, from very hot and arid to the arctic climate. In Canada, there are over two hundred species of wildberries, many of which are edible and grow in highbush or lowbush vegetation [53]. Wildberry harvesting is a Canadian tradition in the latter part of summer for the rural and northern residents who use the wildberries to make jellies, jams and wines. Also, a small seasonal income is created by selling the wildberries to tourists. Aboriginals have utilized wildberries for their nutritional and medicinal value for generations [2]. In North America, there are a wide range of berries that are important parts of tradition ecological knowledge such as salmonberries (*Rubus* spectabilis) which are in the same genus as the raspberry. In indigenous arctic tribes, the salmonberries have been used for health remedies as wound healing and gynecological aids [54,55]. Another example of indigenous use is using the fruit of the wildberries for its antiseptic properties to treat inflammations and infections, to help combat scurvy and urinary tract infections [56]. The composition and content of the phenolic compounds in wildberries fluctuate extensively based on the cultivator, the climate, the season and the growing location [57,58]. Therefore, it is difficult to provide specific data related to the phenolic content in wildberries. However, there is some data which demonstrates that lowbush wildberries contain a higher amount of total phenolics and anthocyanin concentrations [57,59-62]. Wildberries that grow in a cold northern climate with a short vegetation season are characterized by higher amounts of phenolic compounds [63,64]. On the Basse-Côte-Nord, there are many species of wildberries and plants which known to exhibit many of these properties, where the compounds can be extracted and used for other purposes other than food products [1]. Among all the species of berries and plants, there is currently only five species of wildberries which are being analyzed for their potential in extracts and the crowberry has one of the highest amounts of antioxidant activity analyzed thus far.

1.3 The crowberry

1.3.1 Crowberry climate

The crowberry is a small, shiny and round fleshy fruit that appears black in color, but when the skin is broken, the fleshy part of the berry is a deep violet. They are part of the *Ericaceae* family which are a family of flowering plants [65,66]. This family of plants is a lowbush type of evergreen shrub known as a heath which grows in acidic and infertile growing conditions and characterized by open, low-growing woody vegetation located mostly in the northern hemisphere particularly in Scandinavia, Russia and Canada [67]. The Basse-Côte-Nord provides some of the most optimal climates and growing conditions for the crowberry where the berries even grow on small islands surrounded by salt water and virtually no protection from the earth's elements. And with the extremely large surface area of Basse-Côte-Nord that remains mostly untouched by man, there are likely vast abundances of crowberries available that have never been seen to date.

1.3.2 Current uses

Currently in North America, the crowberry is generally used for food products such as jams, jellies, wines and is often in mixtures with other berries, however it is not exploited in commercial products to a large scale. Locally on the region, it is hand-picked and eaten fresh, and/or frozen where it is used throughout the winter in other food products. In some botanical literature, the crowberry has been classified as inedible [66]. This is due to the high amount of tannins in the berry which give it an acidic, bitter and astringent taste which is why it is preferred to be used in products [2,66]. In Finland, the crowberry is the third largest wildberry crop after the lingonberry and the bilberry [2]. Additionally, wines made with the crowberry appear to have exceptional antioxidant activity when in comparison to wine made with red grapes [67]. In recent years, the phenolic compounds, which are known to be present in the crowberry, has been attracting attention for its potential to help prevent and treat many oxidative stress-related diseases such as cardiovascular and neurodegenerative diseases [46,68,69]. The Tanaina,

a tribal group near Anchorage Alaska have used the leaves and stems of the crowberry to treat diarrhea and it is known to aid with kidney trouble [54,70,71].

1.3.3 Whole crowberries and its skin and seed residues

While much of the published research use lyophilized whole crowberries for their research, other research has suggested that much of the phenolic compounds are located in the peel or "skin" of the berries as mentioned previously, the phenolic compounds such as the anthocyanins are located in abundance in the sub-epidermis [2,72]. Some studies also elude to berry seed oil and its composition where it's stated berry seed oil contains n-3 and n-6 polyunsaturated fatty acids which also may have a role in preventing heart disease by lowering triglycerides and reducing blood clotting [48,72,73]. A polyunsaturated acid, γ-linolenic acid is important because it helps reduce body fat content and facilitates β-oxidation in the liver [72,74-76]. Currently, γ-linolenic acid is only sourced from a few plants for commercial purposes including evening primrose (*Oenothera* beinnis) and borage (*Borago* officinalis) [72]. However, the black currant (*Ribes spp.*) is proving to be a rich source of γ-linolenic acid, which is a similar berry to the crowberry, and due to the health benefits, more sources are required. The Lower North Shore Bioproducts Solidarity Cooperative had produced organic waste from the production of their crowberry fruit purée in the form of berry skins and seeds. Figure 1.5 from a report done by Biopterre suggests that the whole berries and residues have very similar quantities of total phenolic compounds, however there is a slightly greater quantity of monomeric anthocyanins in whole berry than the residues. Nonetheless, there is still a relatively high amount of anthocyanin compounds and can therefore likely be extracted and used in commercial products [58,77].

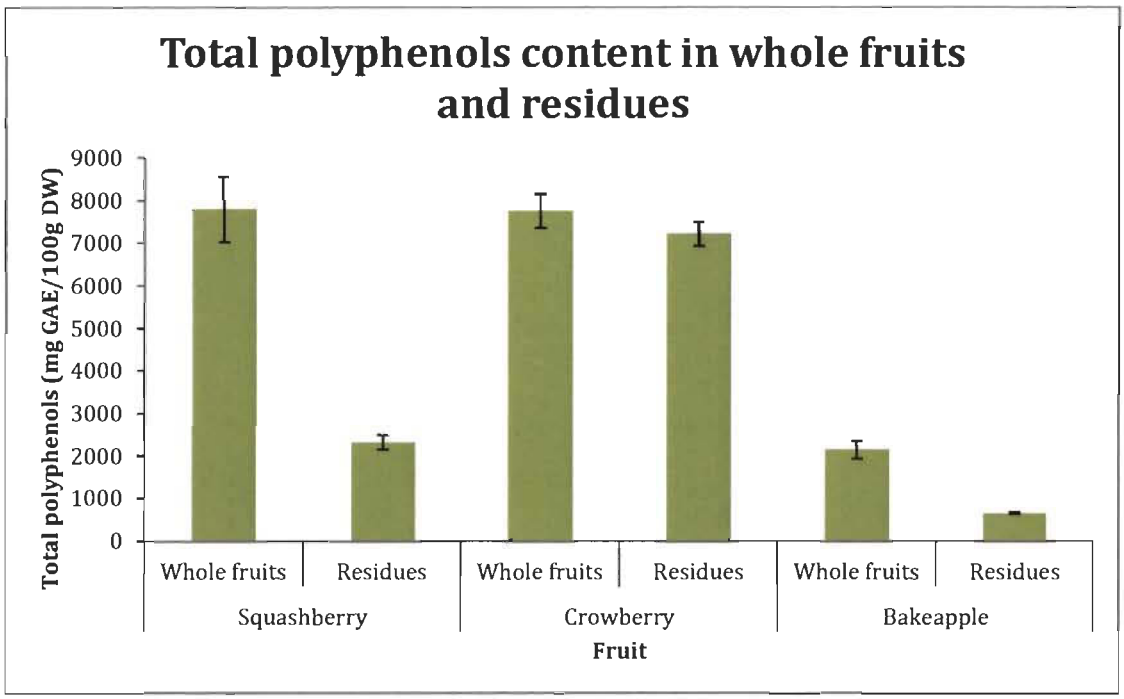


Figure 1.5 Total polyphenols content under dry basis in whole fruits and food processing residues crowberry, squashberry and bakeapple [77].

1.3.4 Antioxidant properties

Crowberries have a remarkable amount of antioxidant activity when compared to other common berries [63,67] as seen in Figure 1.6. When the crowberry is compared against other Canadian northern wildberries, the crowberry has the highest amount of total phenolic content at 503 mg/100 g FW (fresh weight) which is almost four times as high as the other berries analyzed [63]. The quantities of anthocyanins are at similar levels to that of the bilberry which is believed to have the highest anthocyanin content among all the berries [67].

berries	total anthocyanins[a]	antioxidant potencies[b,c] by DPPH	ABTS	FRAP
bilberry	38.8	61 ± 0.3	38 ± 0.3	193 ± 1.1
blackberry	10.1	51 ± 0.4	26 ± 0.4	124 ± 1.0
blackcurrant	21.2	52 ± 1.0	31 ± 0.1	131 ± 4.3
blueberry	10.2	28 ± 0.6	14 ± 0.1	56 ± 2.3
cranberry	4.8	36 ± 0.4	23 ± 0.3	94 ± 1.3
crowberry	41.8	90 ± 0.4	64 ± 0.7	317 ± 1.9
mulberry	16.1	40 ± 0.4	17 ± 0.2	89 ± 0.3
raspberry	10.3	46 ± 0.5	23 ± 0.2	94 ± 1.7
redcurrant	2.5	50 ± 0.4	27 ± 0.8	95 ± 0.2
strawberry	5.2	25 ± 0.1	11 ± 0.2	40 ± 0.3
trolox		47 ± 0.4	20 ± 0.1	

[a] mg/g extract. [b] Berry extracts (2 mg/mL) and trolox (0.1 mg/mL) were used for each method. [c] Antioxidant potencies were indicated as % of radical quenching activity (DPPH and ABTS) and mg trolox equivalent/mL (FRAP) as described in the Materials and Methods.

Figure 1.6 Antioxidant potency and total anthocyanidin content [67].

Figure 1.6 demonstrates that of eleven berries analyzed, the crowberries had the strongest antioxidant activity in multiple types of antioxidant assays. Additionally, the total content of flavonols, which includes quercetin, myricetin and kaempferol was higher than most of the commonly consumed fruits and vegetables with the exception of kale, onion and broccoli [67]. Studies suggest that the crowberry has thirteen types of anthocyanins which are all based on the five common anthocyanidin structures as seen in Figure 1.4. The major anthocyanins present were cyanidin-3-galactoside, delphinidin-3-galactoside and peonidin-3-glucoside/malvidin-3-galactoside where the quantities extracted were 8.04, 8.62 and 10.37 mg/g extract respectively [67]. Therefore, although the crowberry is relatively underutilized today, it holds a great future for potential applications in the food, cosmetic and pharmaceutical marker due to its high and wide variety of phenolic content [58,78].

Although the current studies suggest that the crowberry has high polyphenolic content and antioxidant potential, the literature lacks the connection between the crowberries and the isolation of its general growing location. In Canada, the crowberry naturally grows and flourishes in northern regions, however these regions come with extensive shortcomings in terms of transportation such as lack of road access, sometimes

no road access which leads to major difficulties transporting samples, products and equipment. Also due to the isolation of these regions, there is little access to proper disposal of chemical waste. Therefore, although the research suggests the crowberry has high antioxidant potential and could be used in commercial products, it is important to also consider the circumstances of the regions where these berries would be sourced.

1.4 Objectives and Hypotheses

Given the abundance of crowberries in the Basse-Côte-Nord region and the growing need of finding natural phenolics compounds antioxidants for commercial products, it is indeed necessary to further study the crowberry to conclude the presence of phenolic compounds and antioxidant activity. Simultaneously, to determine these compounds can be extracted using basic techniques, equipment and solvents that would be accessible for the Basse-Côte-Nord region. Thus, purpose of the research presented is to create crowberry extracts and firstly, analyze the total phenolic content as well as the antioxidant activity by determining the anthocyanin content. To accomplish this, four objectives were kept in mind. The first objective was to prepare and preserve the crowberry samples by techniques that are efficient as to not alter the antioxidant properties and with suitable techniques for the region such as using frozen samples for extracts rather than lyophilisation.

To examine two different extraction methods: solid-liquid extraction (SLE) and microwave-assisted extraction (MAE) to determine the most effective process while keeping in mind the limitations of the region is the second objective.

The third objective is to determine if the skin and seed residues of the crowberry have similar phenolic content and antioxidant activity by replicating the experiments done with the whole crowberry samples. This would allow for the crowberry to be one hundred percent used rather than having skins and seeds as waste from the extractions.

The final objective is to determine if there is enough phenolic content and antioxidant activity present to be potentially used in a cosmetic formulation by determining if it is comparable to other fruits already used in cosmetics and natural health products.

In terms of the hypotheses of the research, it is expected that the crowberry will have significant amounts of total phenolic content and antioxidant activity and thus will be comparable to other fruits such as the blueberry. It is also expected that the skins and seed residues will yield similar results to that of the whole crowberry sample. The MAE method is expected to return similar or higher yields than the traditional solid-liquid extraction method while using less solvent and extraction time.

The originality of this research lies in the innovation of taking a wildberry from a devitalized community seeking economic diversification and potentially turning it into a highly desired commercial product while working around the complex circumstances of the location of the region, which is likely the same circumstances for many of the regions where this berry is found in Canada. Also, this has never been done before in Canada. The crowberries are only present in the more northern parts of Canada and this work has never been undertaken to study and publish it in detail. There are few studies that focus on the composition of the crowberry specifically, and even fewer studies that focus on the composition of the crowberry in Canada and no studies on this subject in this region. In the long term, this research could lead to even further quantification of the crowberry and thus more potential could be realized for the Basse-Côte-Nord region and perhaps more Arctic regions.

CHAPTER II

COMPARING POLYPHENOLIC YIELDS FROM THE CROWBERRY *EMPETRUM NIGRUM L.* ON THE BASSE-CÔTE-NORD DU QUÉBEC VIA SOLVENT AND MICROWAVE-ASSISTED EXTRACTIONS

The content of this chapter is written in the form of a scientific article which was published in the journal "Industrial Biotechnology" in June 2019.

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2.1 Contribution of authors

All the research and work that led to the results presented in this article was carried out by Karla Roberts with the research and laboratory assistance of Jessica Poole, who is also a student in the Master of Environmental Science program in the laboratory of Simon Barnabé and Annabelle St-Pierre, a student in the Master of Cellular and Molecular

Biology program. The authorization of the research, issue, research objectives and the methods in which to complete the objectives was determined by Simon Barnabé (director of research), Agathe Vialle (co-director), Benjamin Boëns (researcher), Amadou Diop (researcher) and Louis-Charles Rainville (Merinov researcher). The writing and design of this manuscript and its contents were completed by Karla Roberts. The final corrections of the manuscript were made by Simon Barnabé.

2.2 Scientific article

Comparing Polyphenolic Yields from the Crowberry *Empetrum nigrum L.* on the Basse-Côte-Nord du Québec via Solvent and Microwave-Assisted Extractions

ABSTRACT

Wildberries are recognized worldwide for their nutrition potential, especially for polyphenols such as anthocyanins and flavonoids that are known as antioxidants. The crowberry, *Empetrum nigrum* found in northern climates is a wildberry that has the potential to be used in natural health and cosmetics products due to its high antioxidant activity. This study is focused on antioxidant active crowberries collected from the Basse-Côte-Nord in the province of Québec. The crowberries were analyzed by techniques that are suitable for a remote region such as the Basse-Côte-Nord which has limited access to chemicals, equipment and transportation. Two different extraction processes were examined: conventional such as solid-liquid extraction and unconventional extraction methods such as microwave assisted extraction. Multiple parameters were tested such as solvent type, solvent-water ratios, liquid/solid ratios, time and temperature. The extracts were analyzed using the Folin-Ciocalteu reagent assay for total phenolic compounds, the pH differential method for total monomeric anthocyanins and the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical method for antioxidant activity. The results from the research indicated that although the ethanol mixtures with water solvent generally gave the highest yield, the water extraction gave a significant yield as well. Results also indicated that compared to the solid-liquid extraction method, the microwave-assisted extraction method allowed for generally smaller liquid/solid ratio (75:1 ml/g compared to 25:1 ml/g with the exception of antioxidant activity) and therefore less solvent. It also allowed for less extraction time (1 to 5 hours compared to 5 minutes).

Keywords: crowberry, polyphenols, anthocyanins, microwave-assisted extraction, antioxidants

INTRODUCTION

Empetrum nigrum L., commonly known as the crowberry, is a small, shiny and round fleshy fruit that appears black in color. It is an evergreen shrub which grows in acidic and infertile growing conditions mostly in the northern hemisphere particularly in Scandinavia, Russia and Canada[1-3]. Studies show that high amounts of antioxidants are present in the crowberry[1,4,5-9]. The total concentration of antioxidant activity in the crowberry was reported to be similar to the black currant and blackberry and greater than the blueberry and raspberry[1,10,12,13]. In addition, the studies demonstrate that the crowberry is a great source of anthocyanins with yields similar to the bilberry which contains the highest amount of anthocyanin of eleven common berries examined[13,14]. Therefore, with high amounts of antioxidants, extracts from crowberries could be utilized in natural health and cosmetic products[15].

Today, most cosmetics and health products companies are looking for alternative ingredients which are derived from natural and organic means to replace the controversial synthetic and petrochemical ingredients[15-18]. For example, European companies such as The Innovation Company use Nordic berries from Finland for some antioxidant components of their cosmetic products[19]. Therefore, given that Nordic berries are already used in Europe and there is research suggesting the crowberry has significant yields of polyphenolics, it indicates the crowberry has potential in cosmetic products as a natural, high-antioxidant extract.

Many fruits such as blueberries, raspberries and grapes display antioxidant properties. Antioxidant compounds have significant and multiple biological effects such as preventative roles in illnesses such as cancer and heart disease[4,20-22]. In recent years, there has been numerous studies that attest to these compounds possessing biological activities with major health benefits such as anticarcinogenic, anti-inflammatory, antihepatotoxic, antibacterial, antiviral, antiallergenic, antithrombotic, antioxidant activities and the prevention of DNA damage[23-34]. An example of this health benefit is the reasonable ingestion of alcohol-free red wine where the phenolic compounds in grapes has been shown to improve the antioxidant status of plasma in humans[4,21,22].

Wildberries have been shown to have the highest quantities of antioxidant activity linked with high phenolic concentrations, especially anthocyanidins. Anthocyanidins are mostly responsible for the red, blue and purple color in vegetables and fruits, especially in berries such as blueberries and raspberries as the glycoside form known as anthocyanins[35]. The composition and content of the phenolic compounds in wildberries can fluctuate extensively based on the cultivator, the climate, the season and the growing location[20]. Therefore, the data related to the phenolic content in wildberries can range significantly. However, there is some data which demonstrate lowbush berries such as the blueberry contain a higher amount of anthocyanins than the highbush blueberry[5,11,20,36-39].

Conventional extraction such as solid-liquid extraction requires the use of more solvent and energy and is time consuming[40,41]. It is a common goal of commercial companies to strive to limit the amount of solvent use, energy and time to be more ecofriendly and non-toxic solvents are also often preconized in the cosmetic industry. An upcoming extraction technique called microwave-assisted extraction (MAE) is based on the moisture within the plant materials[42.43]. The microwave radiation causes high temperatures and pressures which causes degradation of the cellulose and in turn, reduces its mechanical strength[44.45]. Once the moisture in the sample is heated and evaporates, a large amount of pressure on the cell wall causes the swelling of the plant cell and therefore the rupturing of the cell wall and leaching out of the phytochemicals[46-48]. Thus MAE could be an innovative way to extract antioxidants active compounds such as polyphenols with reduced amounts of solvents and time.

Although there is some research on the crowberry and its antioxidant properties, there is little research that takes into account the isolation and resource limitations such as transportation, equipment and people in the northern areas where these berries are usually found. The aim of this study is to perform extracts on crowberries using the conventional and MAE methods using different parameters such as time, liquid to solid ratios and temperature. The parameters will be tested with different solvent-water ratio mixtures and water to find the best methods by comparing the yield difference. In addition, colorimetric assays and an antioxidant assay will be performed to determine if the yield of polyphenolic

and antioxidant compounds is potentially substantial to use in the cosmetic industry by comparing with other studies on polyphenols of fruits already used in cosmetics and health products.

MATERIALS AND METHODS

Sampling

The crowberry samples were obtained from the Lower North Shore Bioproducts Solidarity Cooperative which purchased the berries from local berry harvesters from the municipalities of Bonne Esperance (51°27'34"N, 57°45'18"W) and Harrington Harbor (50°29'59"N, 59°28'59"W) on the Basse-Côte-Nord of Québec and in the municipality of Forteau (51°29'36"N, 56°57'24"W) in Labrador. The area covered by the harvesters in each municipality is approximately 250 square kilometers. Since the crowberries were harvested by local harvesters in the wild, there was no specific sampling field. The berries are generally harvested from August to October each year and were frozen for a minimum of a year as this is the only method of preservation on the Basse-Côte-Nord. However, the method of freezing the berries before extraction is common in other studies and the difference between fresh and frozen berry samples is almost undetectable[49-51]. Once the berries were collected by the Lower North Shore Bioproducts Solidarity Cooperative, the whole berry samples were washed and vacuumed packed and frozen at -20°C until extraction.

Chemicals

Gallic acid and Folin-Ciocalteu reagent was obtained from Sigma-Aldrich (Darmstadt, Germany). Potassium chloride (KCl), sodium acetate (CH_3CO_2Na), sodium carbonate (Na_2CO_3) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma Chemical Co. (St. Louis, Missouri). Hydrochloric acid (HCl) and other organic solvents were obtained from Fisher Scientific (Hampton, New Hampshire).

Sample Extraction

Two types of extraction methods were used: solvent and microwave extractions. Before beginning extractions, the samples were thawed at 4°C for 5 hours and homogenized in a blender for 5 minutes. All following extractions were performed in triplicate.

The solvent extractions were done according to the method reported by Aaby *et al.*[52] with some modifications. Briefly, 1 g of sample was placed in different solvents at liquid to solid ratios of 25:1, 50:1 and 75:1 ml/g. The solvents used were ethanol:water (25%, 50% and 75%, water and water acidified with citric acid to a pH of 2. Each mixture was extracted at 1, 2 and 5 hours at both room temperature of 25°C and 70°C in a water bath. Therefore, for each liquid to solid ratio, 6 samples were analyzed in triplicate. Once the extraction was complete, the supernatant was recovered and stored at -20°C until analysis.

Microwave extractions were done according to the methods reported by Gharekhani *et al.*[53] with some modifications. The microwave extraction system used was the flexiWAVE advanced flexible microwave synthesis platform (Milestone, Sorisole, Italy), which consists of temperature and power control unit, microwave cavity, extraction flask, infrared temperature sensor and glass adapter and condenser. Briefly, 1 g of sample was placed in different solvents at liquid to solid ratios of 25:1, 50:1 and 75:1 ml/g. The same liquid to solid ratios that were used in the conventional solvent extraction were used in the microwave extraction. The power and temperature were set on 700 watts (W) and 70°C respectively. The extraction time was 5, 10 and 15 minutes. Therefore, for each liquid to solid ratio, 3 samples were analyzed in triplicate. Once the extraction was complete, the supernatant was recovered and stored at -20°C until analysis.

Determination of Total Phenolics

The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu assay method reported by Ainsworth and Gillespie[54] with some modifications.

Briefly, 100 µL of the extract was added to 200 µL of 10% volume/volume percent (v/v) Folin-Ciocalteu reagent and 800 µL of 700 mM Na_2CO_3 solution was added. The solution was incubated at room temperature for two hours and the absorbance was read at 765 nm with a Varian Cary 50 Bio UV-Visible Spectrophotometer (Agilent Technologies, Santa Clara, California). The amount of total phenolics was expressed as mg gallic acid equivalents (GAE) per g of dry matter.

Determination of Total Monomeric Anthocyanins

The amount of total monomeric anthocyanin was determined according to the pH differential method reported by Lee *et al.*[55] with some modifications. Briefly, a pH 1 buffer solution was prepared by adding 1.86 g of KCl with 980 ml of distilled water. The pH was adjusted to 1 with concentrated HCl and placed in a 1000 ml volumetric flask and diluted to volume with distilled water. A pH 4.5 buffer solution was also prepared by added 54.43 g of CH_3CO_2Na with 960 ml of distilled water. The pH was adjusted to 4.5 with concentrated HCl and placed in a 1000 ml volumetric flask and diluted to volume with distilled water. Using a 25 ml volumetric flask, the dilution factor was determined by diluting 5 ml of extract with the pH 1 buffer until absorbance at 520 nm is within linear rage (0.2-1.4). Once the dilution factor is determined, two dilutions of the extract were prepared, one with the pH 1 buffer and the other with the pH4.5 buffer. The absorbance was determined for both solutions at 520 nm and 700 nm versus a blank cell filled with distilled water. The monomeric anthocyanin pigments (MAP) concentration, expressed as cyanidin-3-glucoside equivalents, was calculated as follows:

$$\text{MAP (cyanidin-3-glucoside equivalents, mg/L)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l}$$

where A= (A520 nm – A700 nm)$pH_{1.0}$ – (A520 nm – A700 nm)$pH_{4.5}$; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor; l = pathlength in cm; ε = 26 900 molar extinction coefficient, in L x mol^{-1} x cm^{-1}, for cyanidin-3-glucoside; and 1000 = factor for conversion from g to mg.

Determination of Antioxidant Activity

The antioxidant activity was determined according to the DPPH Radical Quenching Assay method reported by Nikolova *et al.*[56] with some modifications. Briefly, 2.5 ml of different concentrations of extracts (10, 20, 50, 100, 200 and 300 μg/ml in 95% (v/v) ethanol) were added to 1 ml of 0.3 mM of DPPH in 95% (v/v) ethanol. The solution was incubated in the dark at room temperature for thirty minutes. The absorbance was measured at 517 nm and converted into the percentage antioxidant activity using the following equation:

$$\text{Scavenging capacity (\%)} = \frac{1 - Absorbance[sample] - Absorbance[blank]}{Absorbance[control]} \text{ x } 100$$

where 1 ml of 95% (v/v) ethanol and 2.5 ml of extract were used as the blank and 1 ml of DPPH solution and 2.5 ml of 95% (v/v) ethanol were used as the control. The concentration (μg/ml) required to scavenge 50% of the DPPH reagent (IC50) was calculated. The extract with the best antioxidant activity is found when 50% of the scavenging activity is measured at the lowest concentration[57].

Statistical Analysis

The analysis results were processed using two-way analysis of variance with the software JMP Pro 11 (SAS, Cary, NC, United States). Differences at $p\text{-}value < 0.05$ were considered to be significant. In addition, significantly different results were processed using Tukey's HSD post hoc test.

RESULTS AND DISCUSSION

Conventional Extractions

Total Phenolic Content

Table 1 indicates the maximum yields obtained at 25°C using a 75:1 ml/g (water, citric acid) and 75% (ethanol) liquid to solid ratio at three different times of 1, 2 and

5 hours for total phenolic content and a 25:1 ml/g (water, citric acid) and 25% (ethanol) liquid to solid ratio for total monomeric anthocyanins. The results depict that for all three solvents, the 5-hour extraction time gave the highest total phenolics at 25°C (Table 1). The total phenolics after 5 hours was 186.53 mg GAE/g using 75% ethanol. Water extracted the lowest amount of total phenolics at 35.98 mg GAE/g using 75:1 ml/g. This result was consistent with another study by Spigno and Faveri, where extraction yields over 5 to 24 hours concluded that water extractions gently increase over time whereas alcohol extracts strongly increase over time[58]. However, in this particular study, when comparing between the 5 and 24 hours, the phenolics became more unstable and fluctuated at 24 hours. In most of the literature referenced for this study, extraction time was rarely more than 2 hours. That is why it was important to examine extraction times that were less and greater than 2 hours because literature implies that active plant compounds could degraded after a longer extraction time resulting in a lower phenolic yield[12,59,60].

Different liquid to solid ratios were examined using the 5-hour extraction time to determine the highest total phenolic content at 25°C. Ethanol extracted more than 4 times more compounds than water or 1% citric acid (Figure 1). The highest total phenolic content was 186.53 mg GAE/g using 75% ethanol and water extracted the lowest at 24.46 mg GAE/g using 25:1 ml/. Most studies used alcohol solvents such as ethanol to extract plant compounds rather than water. One study suggested that using varying solvents with different polarities allowed for a higher accuracy when extracting phenolic compounds and that highly polar solvents such as alcohol solvents had a high effectiveness against antioxidants[61]. At 70°C, the heat slightly increased the total phenolic content with water and citric acid (Figure 1). However, temperature increase favors extraction by increasing solubility of all compounds, not just phenolic compounds therefore it is possible, the increase seen may not just be phenolic content[58]. Also, polyphenols are heat sensible so a 70°C upper temperature limit was chosen based on various temperatures reported in other studies, although some reported 50-60°C is sufficient[62]. Finally, it was observed that 75% ethanol produced slightly better total phenolic content as well as the 75:1 ml/g liquid to solid ratio for water and 1% citric acid,

which was consistent with studies that conventional extractions generally use more solvent in the extraction process[53].

Total Monomeric Anthocyanins

A 2-hour extraction time at 25°C produced slightly higher MAP where the highest amount of anthocyanins extracted was 20.49 mg/g using 25% (Table 1).

Ethanol extracted almost six times more anthocyanin compounds where 20.49 mg/g MAP was extracted using 25% ethanol and 3.49 mg/g MAP was extracted using water at 25:1 ml/g liquid to solid ratio at 25°C (Figure 2). The 75:1 ml/g water extraction extracted the least amount at 1.43 mg/g MAP at 25°C. The lower solvent liquid to solid ratio extracted better than the higher solvent liquid to solid ratio which implies that to extract anthocyanins in the conventional method, less solvent can yield higher amounts of anthocyanins. Unlike the phenolic content analysis, the increase in temperature to 70°C generally produced a slightly lower yield of anthocyanins than 25°C (Figure 2) which indicates the anthocyanins specifically could be degraded under higher temperatures[59].

Antioxidant Activity

In a study by Phongpaichit et al., they considered that IC50 values of greater than 250 μg/ml is inactive meaning no antioxidants present, 100-250 μg/ml is weakly active, 50-100 μg/ml is moderately active, 10-50 μg/ml is strongly active and less than 10 μg/ml is very strongly active[63]. The antioxidant findings in this section were compared to those ranges of values to have a better understanding of how antioxidant activity is classified.

A 1-hour extraction time at 25°C produced the best highest antioxidant activities for all three of the solvents examined. For water, a 50:1 ml/g liquid to solid ratio produced the best antioxidant activity at 122.26 μg/ml (Figure 3a). The lowest antioxidant activity produced was the 75:1 ml/g liquid to solid ratio after 5 hours at 205.30 μg/ml. There was no significance difference in antioxidant activity between the liquid to solid ratios as all

the antioxidant activity for the water are considered weakly active. The 1% citric acid solvent yielded higher antioxidant activity than water (Figure 3b). The highest amount of antioxidant activity for the 1% citric acid extractions was 84.86 μg/ml using 75:1 ml/g liquid to solid ratio. The ethanol solvent had over five times better yields of antioxidant activity (Figure 3c). The highest amount of antioxidant activity produced was 12.74 μg/ml with a 75% liquid to solid ratio. Therefore, for antioxidant activity, the 1-hour extraction time was optimal because it produced the maximum yields. Also, 25°C generally produced slightly higher yields than 70°C (Figure 3d). This is ideal because a 25°C extraction time requires less time and energy demand and thus suggesting that it is not necessary or optimal to heat the extractions. There is also no significant difference between 70 and 25°C.

The conventional extraction methods exhibit that the crowberries of the Basse-Côte-Nord have strong phenolic and antioxidant activity. One study stated the total phenolic content in black currant marc was 9.72 mg/g total polyphenols and 6.80 mg/g anthocyanins after 24 hours compared to the 186.53 mg GAE/g phenolic content and 20.49 mg/g anthocyanins obtained in 5 hours in this research[64]. Another study reports that the crowberry has almost twice the antioxidant potency as the bilberry[1] which is a high-antioxidant berry. This research showed that the crowberries of the Basse-Côte-Nord had higher yields than other studies therefore may have a greater potential in products. This could be due to the land being remote and 'untouched by man', proximity to the ocean or a number of other factors that could be researched further.

Microwave-Assisted Extractions

Total Phenolic Content

Table 1 indicates the maximum yields obtained at 70 °C using a 25:1 ml/g (water, citric acid) and 25% (ethanol) liquid to solid ratios at three different times of 5, 10 and 15 minutes for total phenolic content and total monomeric anthocyanins. For all three solvents, the 5-minute extraction time produced the highest total phenolics (Table 1).

Different liquid to solid ratios were examined at the 5-minute extraction time to determine the highest total phenolic content at 70°C. Ethanol extracted higher phenolics at 226.41 mg GAE/g using a 25% ethanol liquid to solid ratio and the lowest phenolics yielded were 35.03 mg GAE/g using 75:1 ml/g 1% citric acid (Figure 4). From previous studies, MAE generally reduced the liquid to solid ratio and extraction time which is the case with the results yielded from these extractions. For example, the conventional extraction methods used 75% ethanol for example to get 186.53 mg GAE/g in 5 hours and MAE used 25% ethanol to get 226.41 mg GAE/g in 5 minutes.

Total Monomeric Anthocyanins

A 5-minute extraction time yielded the highest amounts of anthocyanin pigments for ethanol at 20.81 mg/g, 8.07 mg/g for citric acid and 9.72 mg/g for water (Table 1).

With the 5-minute extraction time, the results indicate that ethanol extracted two times more anthocyanins than the other solvents using 25% ethanol at 20.81 mg/g and the lowest amount extracted was 3.43 mg/g using 75:1 ml/g citric acid (Figure 5). The anthocyanin yields for the conventional extractions were 3.49, 8.38 and 20.49 mg/g for water, citric acid and ethanol respectively which were very similar yields suggesting that the MAE did not extract more anthocyanins, but the time was greatly reduced to get a similar result.

Antioxidant Activity

The 5-minute extraction time produced the highest antioxidant activity for all three solvents. For water, the liquid to solid ratio that yielded the highest antioxidant activity was 50:1 ml/g at 47.89 μg/ml (Figure 6a). The MAE method improved antioxidant extraction as the conventional method with water yielded 122.26 μg/ml in 1 hour and the microwave method with water yielded 47.89 μg/ml in 5-minutes. The results demonstrate overall higher antioxidant activity using 1% citric acid as a solvent than water; 46.09 μg/ml was yielded using 75:1 ml/g liquid to solid ratio (Figure 6b).

The results illustrate that ethanol was the most ideal solvent and using a liquid to solid ratio of 75%, it gave an antioxidant activity yield of 7.85 μg/ml which is considered very strongly active (Figure 6c). Therefore, the extraction time is greatly reduced which agreed with other studies that the MAE method had reduced extraction times when compared to more traditional extraction methods[64,65].

Although the microwave greatly reduced extraction time, it did not always yield the highest results with the lesser amount of solvent. In the case of antioxidants, more solvent was required to get the highest yields, however the 25:1 ml/g water and 1% citric acid and 25% ethanol are still comparable to the highest yields and higher than the yields from the conventional methods as well and could be potentially used for products. The optimal microwave extraction time of 5 minutes in this research was not consistent with other studies which report optimal times of 10-15 minutes[53,66]. It is unknown why this time difference occurred; however, it may be due to the different microwave apparatus. It was suggested by one study, the microwave method could increase yields by 20%, however the yields generally did not increase by 20% in this research which could also be due to the microwave apparatus used[66].

In general, the statistical results were considered significant if $p < 0.05$. The results from this study indicate that the ethanol solvent was significantly better than citric acid and water for both extractions methods. When comparing the two extraction methods, statistics proved a significant difference in extraction times. For the conventional extraction method, statistics proved a significant difference between liquid to solid ratios where the higher ratio generally produced higher yields and for the unconventional extraction method, statistics proved there was a significant difference between liquid to solid ratios where a lower ratio produced higher yields. Statistics also proved there was no significant difference between the two temperatures examined for the conventional method.

CONCLUSION

Overall, the results from the conventional and MAE methods research does generally correlate with other studies found comparing the two methods. MAE gave similar or higher yields using less solvent and the extraction time is greatly reduced. Ethanol proved to be the highest yielding extraction solvent which was expected as in most studies it is the traditional standard solvent to which other solvents are compared[61,67].

To conclude, this research exhibits that there is phenolic and antioxidant activity in the crowberry from the Basse-Côte-Nord and it is comparable to other fruits that are considered high in antioxidants[1]. As expected, ethanol did extract the highest yields of total phenolics, anthocyanins and antioxidant activity. The water and citric acid, which are more cosmetic-friendly and easier to implement on the Basse-Côte-Nord, did not extract as well as ethanol. Nevertheless, this research demonstrates that the crowberry indeed has extractable phenolic compounds. Furthermore, it would be valuable to further research other cosmetic-friendly solvents with the improved MAE methods to determine if higher yields are possible. There is also the necessity to analyze variability of the species in the region by working on a traceable batch from a targeted harvesting zone to compare with these first polyphenolic screenings. To further this idea, it would also be beneficial to determine if the crowberry could be cultivated for agricultural production. Although the crowberry is present on the Basse-Côte-Nord in abundance, it is classified as a wildberry in this region at the moment and there is no guarantee of a consistent supply. Thus, a crucial consideration for using crowberries in products is to ensure the consistent availability of the resource. Finally, additional work is underway to confirm the polyphenolic presence in these extracts by further quantifying and purifying the compounds clearly present in the promising northern crowberry to potentially turn them into a commercial product.

ACKNOWLEDGMENTS

This work was financially supported by MITACS, the Lower North Shore Bioproducts Solidarity Cooperative and the Coasters Association Inc. The authors are grateful to Benjamin Boëns, Isabel Desgagné-Pénix, Nathalie Boudeau, Julien Bley, Kokou Adjallé and Alain Tremblay for their assistance.

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Figure 4 Total phenolic content (mg GAE/g) after a 5-minute extraction time with liquid to solid ratios of 25:1 ml/g to 75:1 ml/g for water and 1% citric acid and 25% to 75% for ethanol at 70 °C using the MAE method. Vertical bars indicate SD calculated among triplicate samples.

Figure 5 Total monomeric anthocyanins (mg/g) after 5-minute extraction time with liquid to solid ratios of 25:1 ml/g to 75:1 ml/g for water and 1% citric acid and 25% to 75% for ethanol at 70 °C using the MAE method. Vertical bars indicate SD calculated among triplicate samples.

Figure 6a Antioxidant activity (μg/ml) of extracts with water as solvent using extraction times of 5 to 15 minutes and liquid tol solid ratios of 25:1 to 75:1 ml/g at 70°C using the MAE method. Vertical bars indicate SD calculated among triplicate samples.

Figure 6b Antioxidant activity (μg/ml) of extracts with 1% citric acid as solvent using extraction times of 5 to 15 minutes and liquid to solid ratios of 25:1 to 75:1 ml/g at 70 °C using the MAE method. Vertical bars indicate SD calculated among triplicate samples.

Figure 6c Antioxidant activity (μg/ml) of extracts with ethanol as solvent using extraction times of 5 to 15 minutes and liquid to solid ratios of 25 to 75% at 70°C using the MAE method

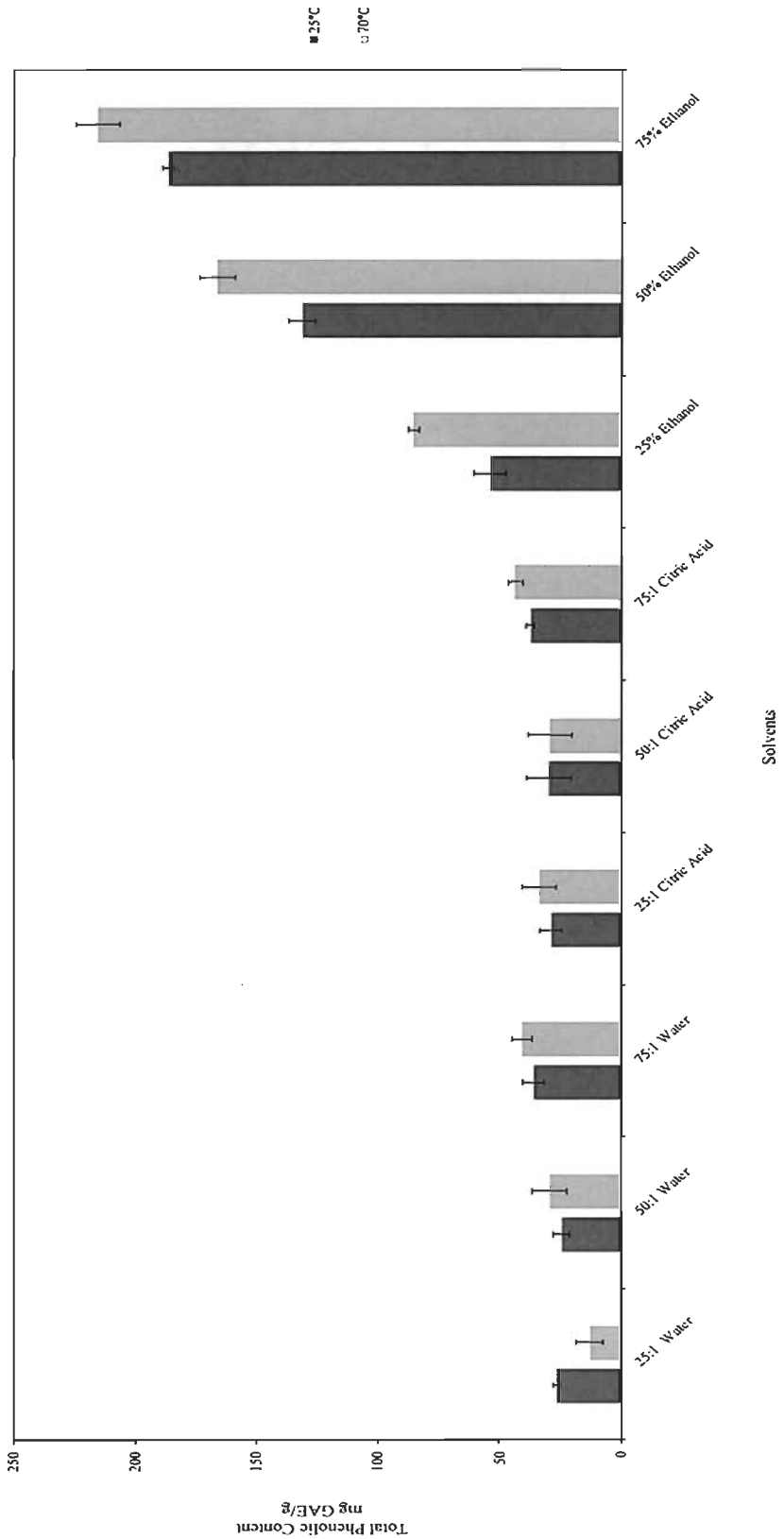
25°C
70°C
Total Phenolic Content
mg GAE/g
250
200
150
100
50
0
75% Ethanol
50% Ethanol
25% Ethanol
75:1 Citric Acid
50:1 Citric Acid
25:1 Citric Acid
75:1 Water
50:1 Water
25:1 Water
Solvents

Figure 1.

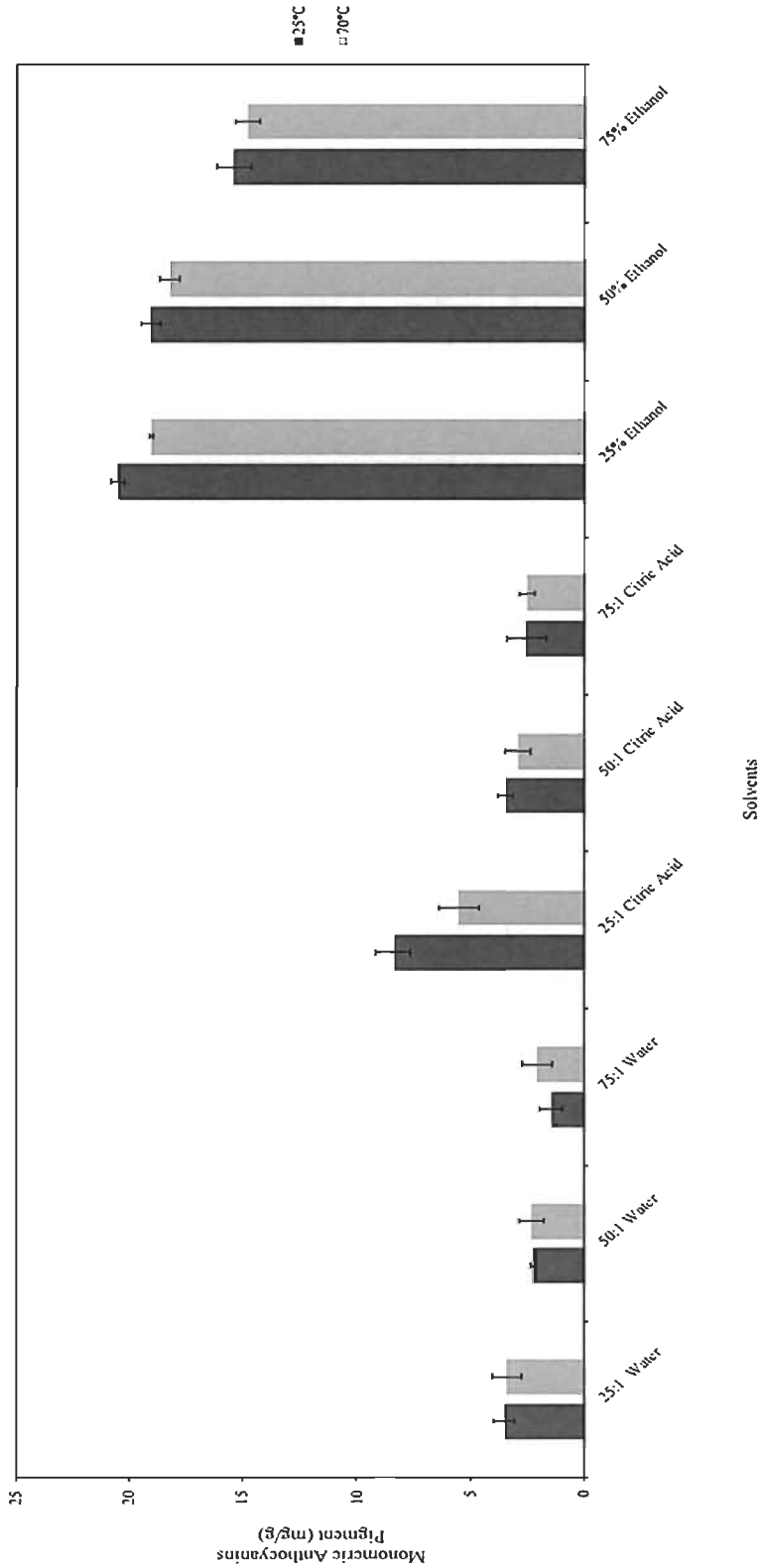
■25°C
□70°C
Monomeric Anthocyanins Pigment (mg/g)
0
5
10
15
20
25
25:1 Water
50:1 Water
75:1 Water
25:1 Citric Acid
50:1 Citric Acid
75:1 Citric Acid
25% Ethanol
50% Ethanol
75% Ethanol
Solvents

Figure 2.

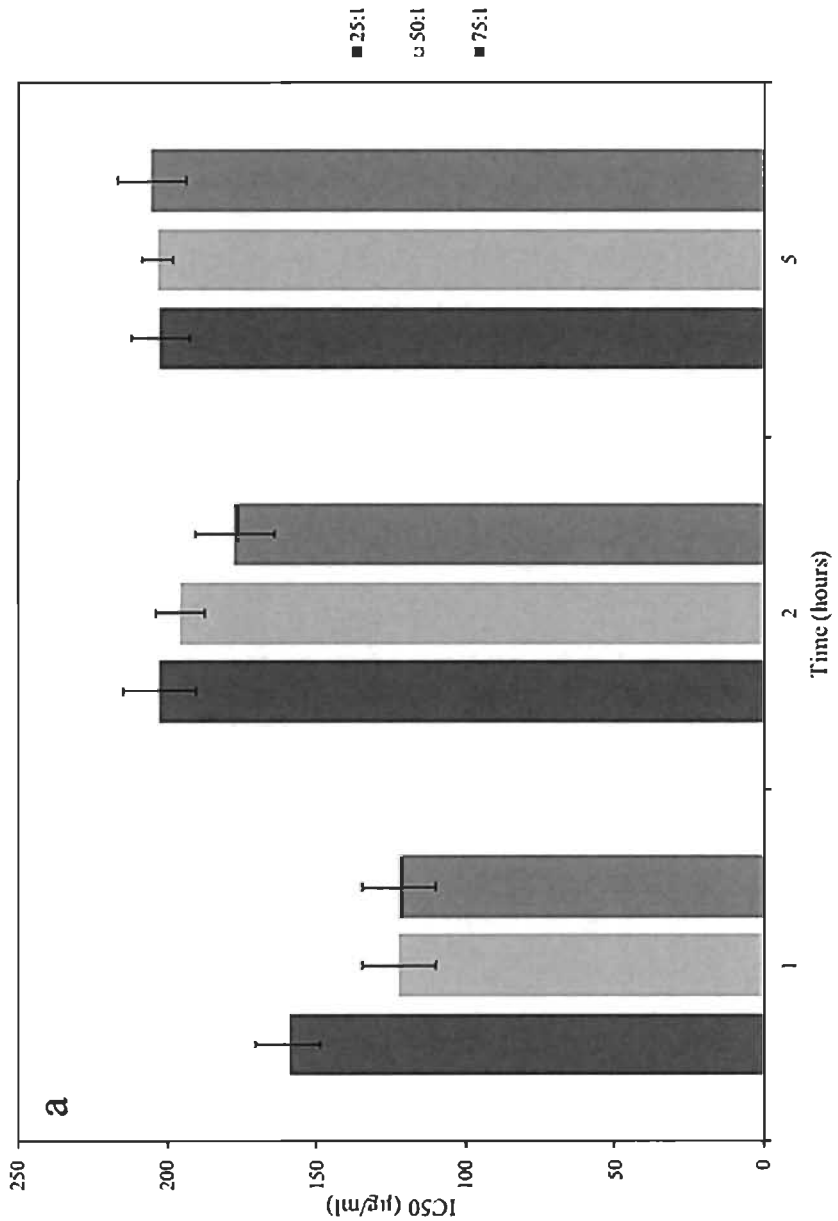

Figure 3a.

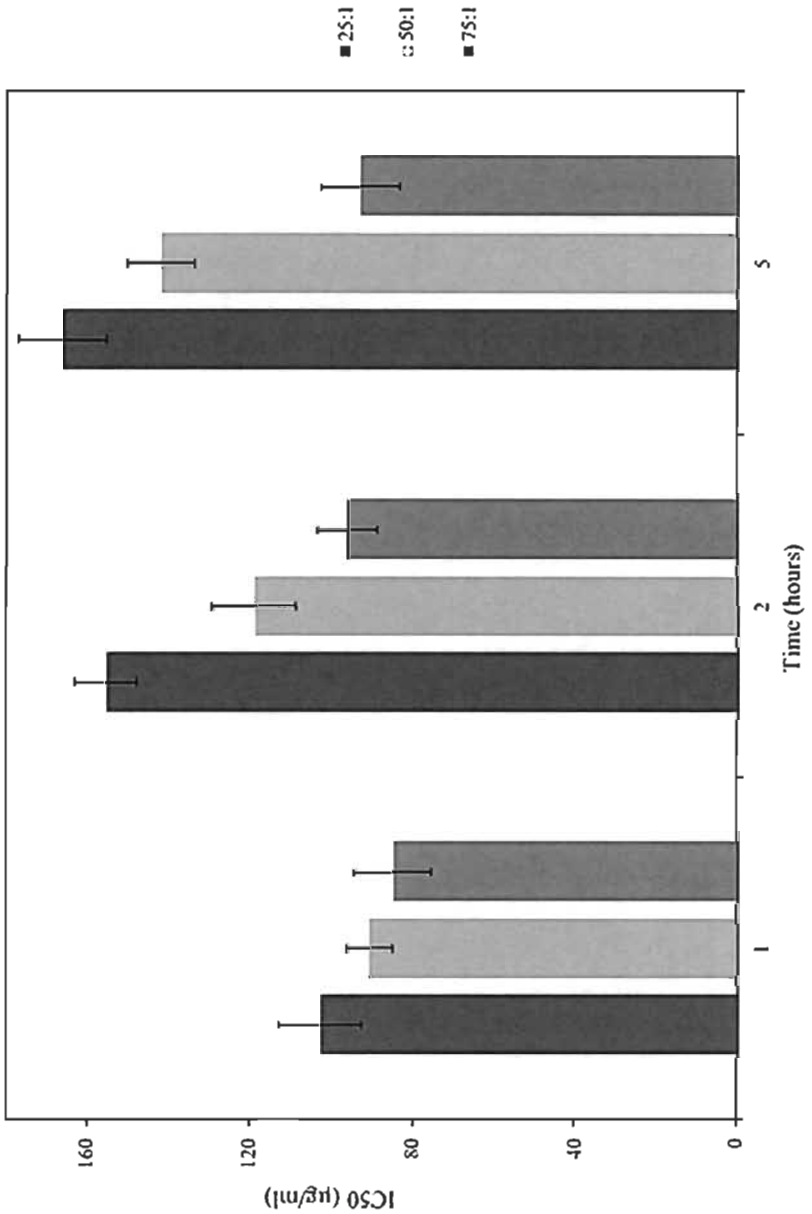

Figure 3b.

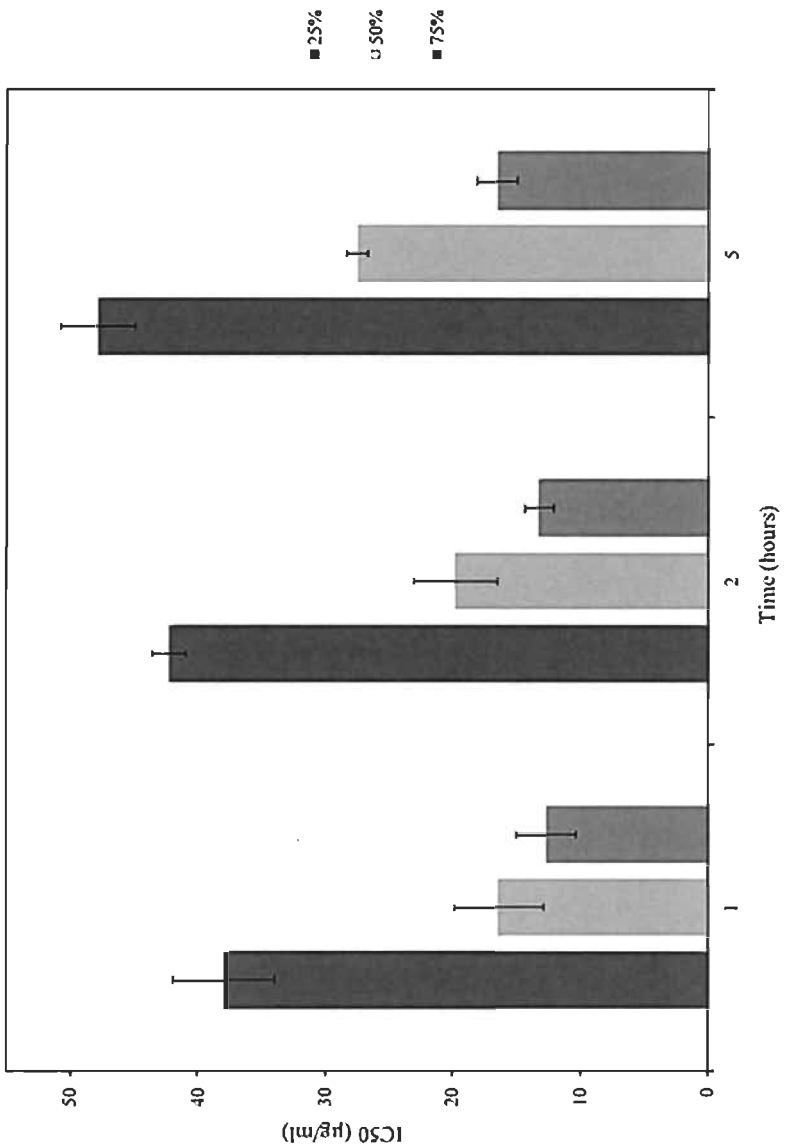

Figure 3c.

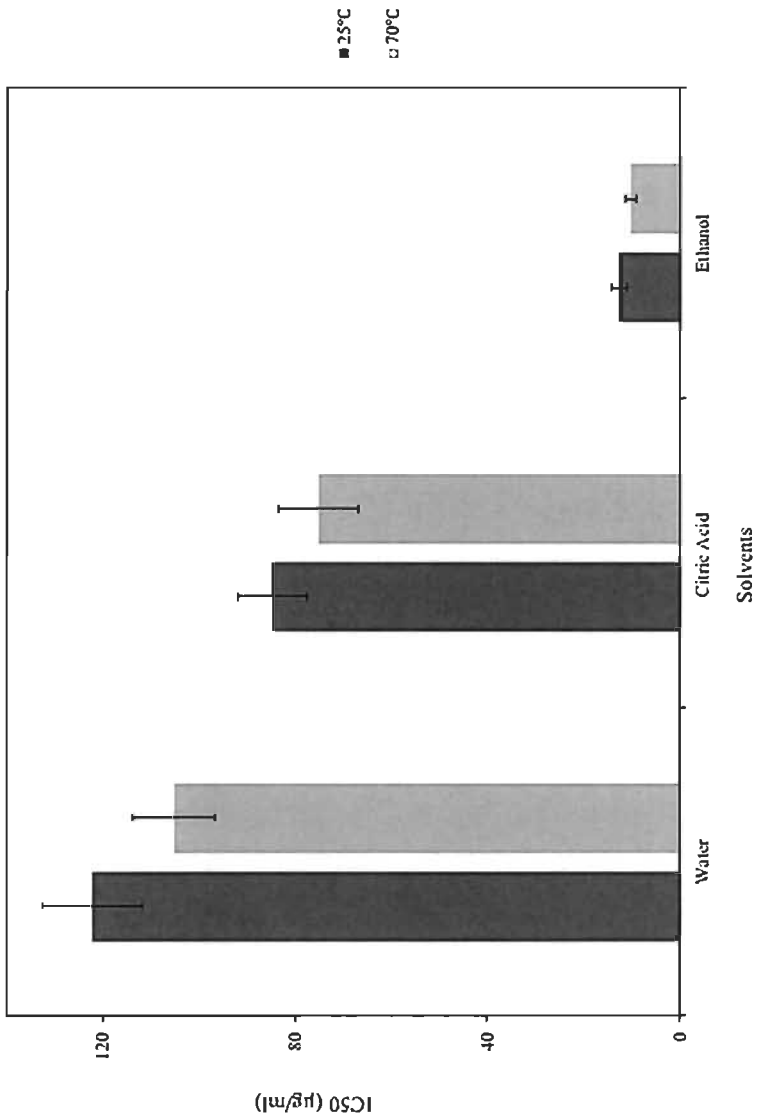
■ 25°C
□ 70°C
IC50 (µg/ml)
120
80
40
0
Water
Citric Acid
Ethanol
Solvents

Figure 3d.

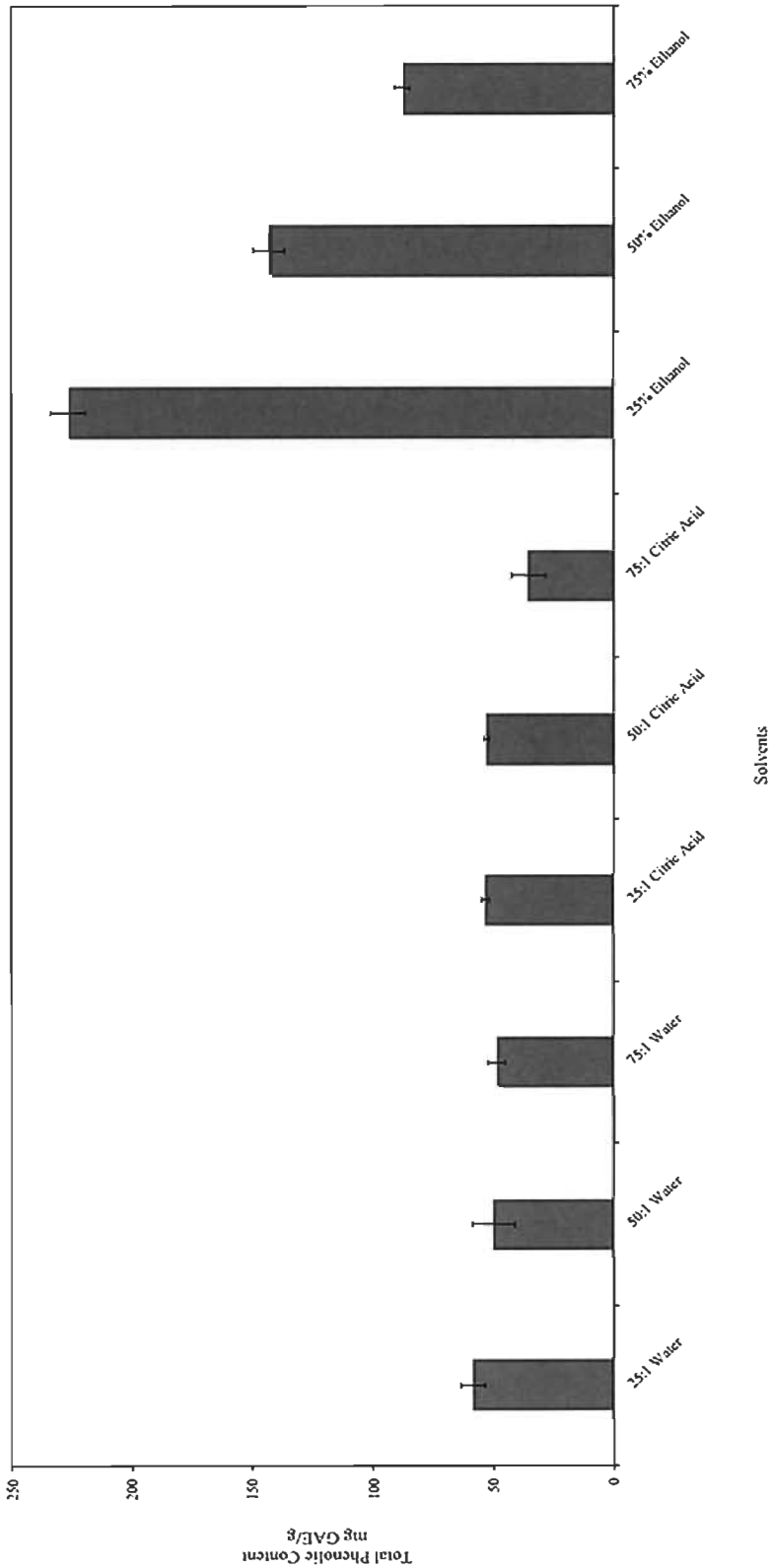
Total Phenolic Content
mg GAE/g
250
200
150
100
50
0
25:1 Water
50:1 Water
75:1 Water
25:1 Citric Acid
50:1 Citric Acid
75:1 Citric Acid
25% Ethanol
50% Ethanol
75% Ethanol
Solvents

Figure 4.

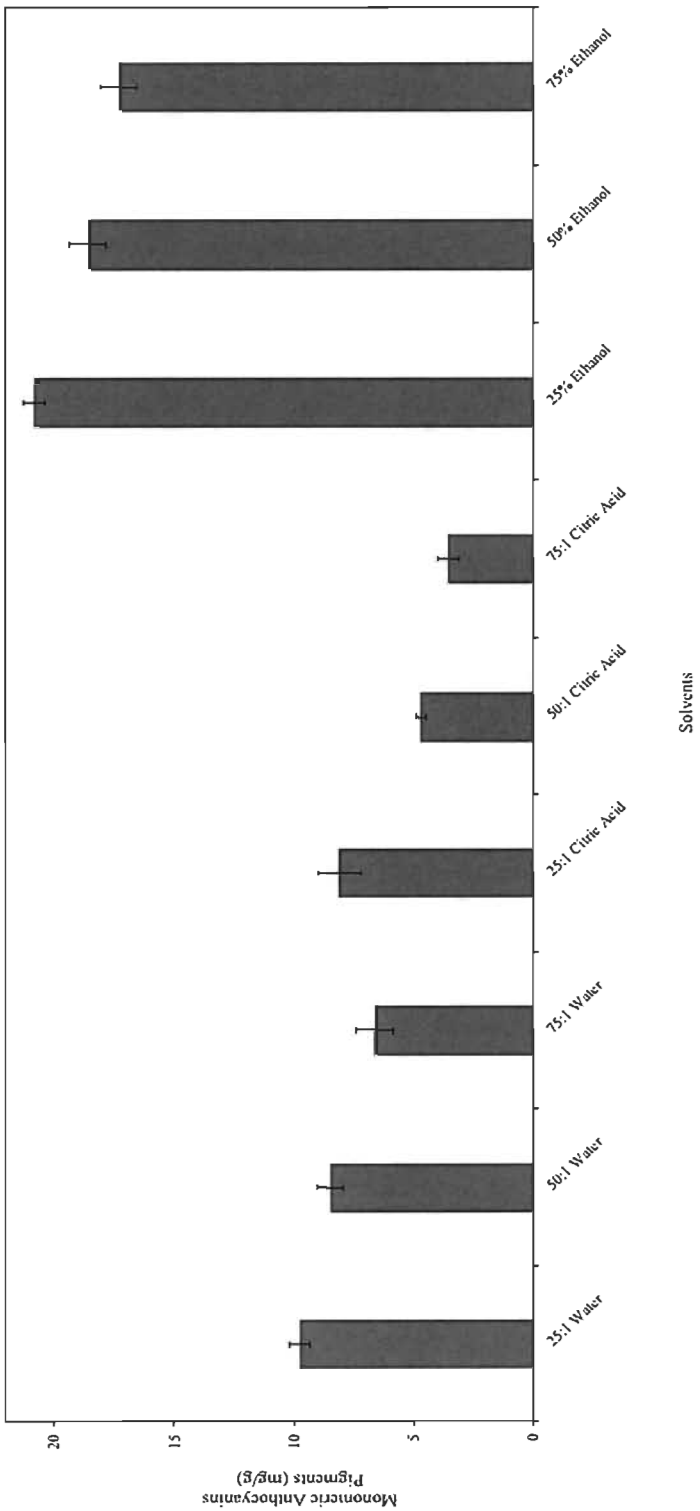
Monomeric Anthocyanins
Pigments (mg/g)
0
5
10
15
20
25:1 Water
50:1 Water
75:1 Water
25:1 Citric Acid
50:1 Citric Acid
75:1 Citric Acid
25% Ethanol
50% Ethanol
75% Ethanol
Solvents

Figure 5.

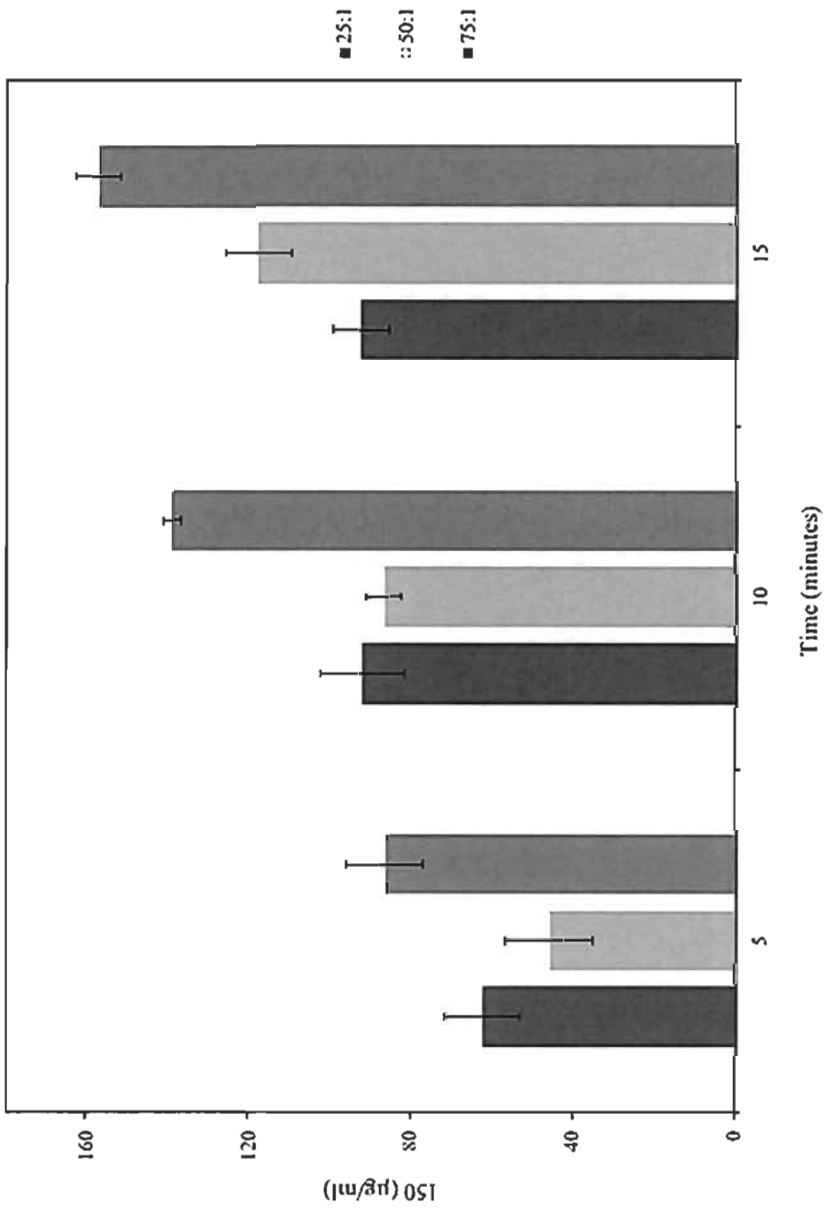

Figure 6a.

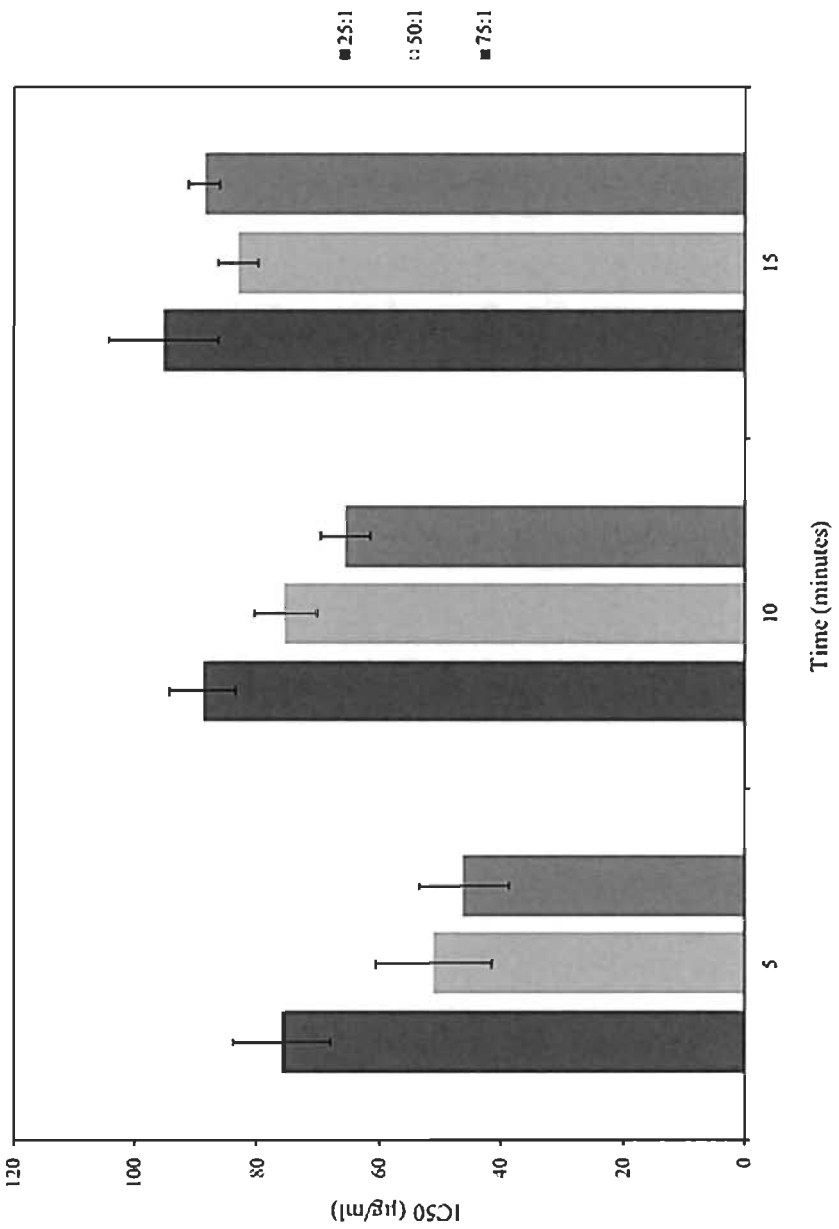

Figure 6b.

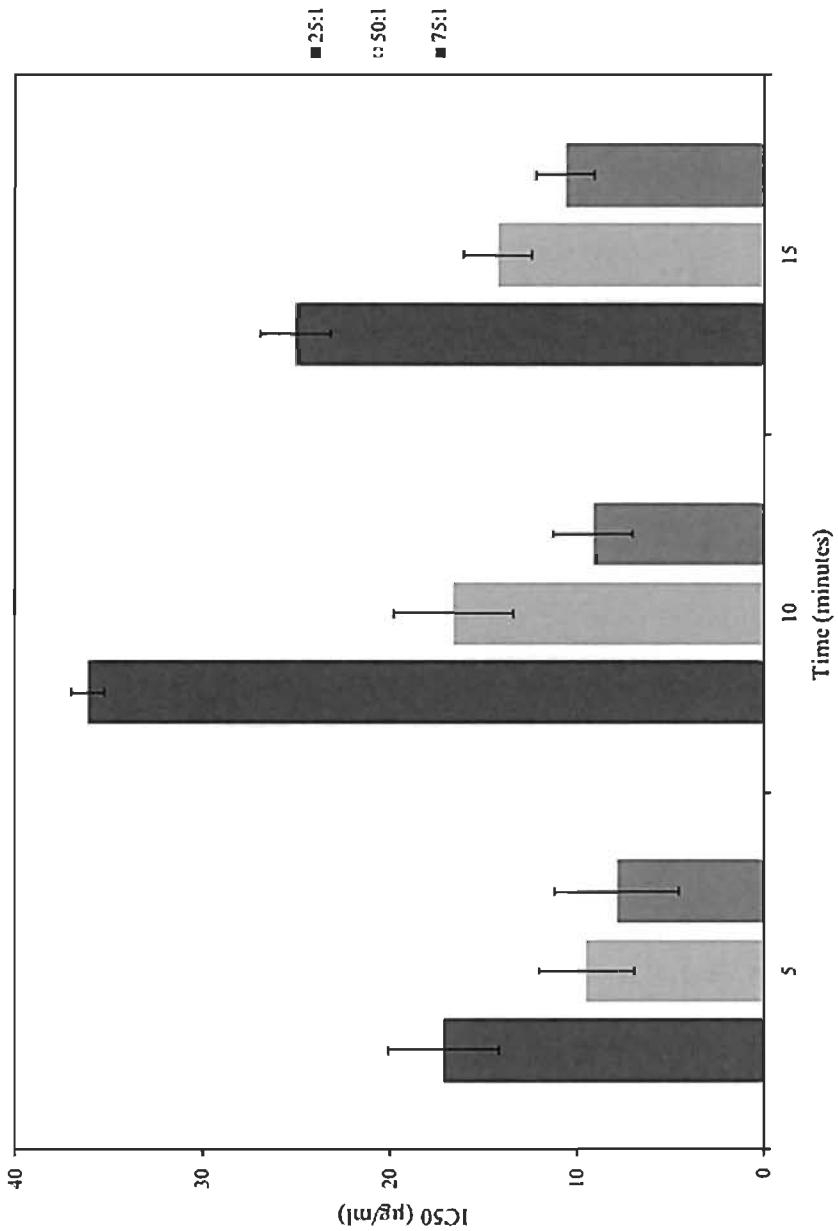

Figure 6c.

Table 1. Total phenolic content (mg GAE/g) and total monomeric anthocyanins (mg/g) at 25°C using an extraction time of 1 to 5 hours with a liquid to solid ratio of 75:1 ml/g for water and 1% citric acid and 75% ethanol using conventional extraction methods and total phenolic content and total monomeric anthocyanins at 70°C using an extraction time of 5 to 15 minutes with a liquid to solid ratio of 25:1 ml/g for water and 1% citric acid and 25% for ethanol using the MAE method.

		Total Phenolic Content mg GAE/g			**Anthocyanins mg/g**		
Conventional Solid-Liquid Extractions	**Time**	Water 75:1	1% Citric Acid 75:1	Ethanol 75%	Water 25:1	1% Citric Acid 25:1	Ethanol 25%
						25°C	
	1 hour	20.54	33.32	133.53	2.91	7.25	19.60
	2 hours	23.53	37.11	131.49	3.49	8.38	20.49
	5 hours	35.98	37.61	186.53	2.43	6.42	17.67
Unconventional Microwave-Assisted Extractions		Water 25:1	1% Citric Acid 25:1	Ethanol 25%	Water 25:1	1% Citric Acid 25:1	Ethanol 25%
						70°C	
	5 mins	57.88	52.86	226.41	9.72	8.07	20.81
	10 mins	43.33	52.32	155.28	6.78	7.31	19.72
	15 mins	42.12	45.89	157.59	6.51	6.57	18.19

SUPPLEMENTARY DATA

Table S1. Total phenolic content (mg GAE/g) and total monomeric anthocyanins (mg/g) at 70°C using an extraction time of 1 to 5 hours with a liquid to solid ratio of 75:1 ml/g for water and 1% citric acid and 75% ethanol using conventional extraction methods.

		Total Phenolic Content mg GAE/g			**Anthocyanins mg/g**		
Conventional Solid-Liquid Extractions	**Time**	Water 75:1	1% Citric Acid 75:1	Ethanol 75%	Water 25:1	1% Citric Acid 25:1	Ethanol 25%
						70°C	
	1 hour	25.26	41.98	137.23	2.05	5.12	18.79
	2 hours	29.31	40.79	153.04	3.35	5.50	19.01
	5 hours	40.64	43.35	210.24	1.71	4.95	16.98

CHAPTER III

DISCUSSION

3.1 Return to the problem and results

As mentioned earlier in this study, the Basse-Côte-Nord region of Québec is facing economic devitalization due to the fact that the region has heavily relied on the fishing industry for generations and over time this industry has severely declined likely due to years of overfishing. And now with the changing climate and global warming, it will make this resource continually unreliable because it is unknown how the effect of climate change will affect the fish and crustacean species. Thus, there is a desperate need in the along entire region to establish another industry for the survival of the communities. The approach that was taken to do this was to consider the other natural resources in the area such as algae and wildberries, like the crowberry. Presently, there is very little research on the crowberry, its composition and benefits, especially in North America. Majority of the current studies are performed on berry samples from Europe where they are more established and advanced in exploiting the wildberries. The crowberry is interesting because it is well documented that generally the darker the berry, the higher the antioxidant components and the crowberry is indeed very dark in color. Therefore, there is an urgency to study the resources such as the crowberry, so they can be developed into products for the nutraceutical and cosmeceutical sectors.

Simon Barnabé's laboratory, its researchers and their partner researchers from CCTTs such as Biopterre and Merinov, created a team and first investigated the possibility of studying the plants from the list of plant species available on the Basse-Côte-Nord region. The wildberries were given priority because of the published studies indicating that berries are known to have anthocyanins and other phenolic compounds which have a range of anti-cancer and heart disease properties which include antioxidant, anti-inflammatory and cell regulatory effects [26-28]. In a report done by Biopterre for

the Lower North Shore Bioproducts Solidarity Cooperative, it indicated that out of the three wildberries from the region examined, the crowberry had one of the highest total phenolic content and three times most anthocyanins than the other berries [77]. Also, the wildberries are generally readily available as they are already harvested and sold by the local people, thus they are frozen in stock. Since there are few studies specifically on the crowberry, but the existing studies such as Ogawa *et al.* (2008), it is found to be one of the most antioxidant-rich berries, and comparable with other fruits already on the market [2,31,66,67]. Therefore, the crowberry was the berry of choice for this project.

The predominant purpose and objective of this research was to create a crowberry extract and determine the total phenolic content and the antioxidant activity. Although other studies asses the crowberry and its phenolic compounds, our study focuses more the commercial potential for a crowberry extract with the concept that the extract will be created on the region where access to equipment, chemicals and transportation will be a matter of contention. Additionally, this has also been carried out with the approach that the extracts will be used in cosmetic products therefore the types of additives that can be used in the extract are limited notably even more so with the further limitation of the eventual location. Therefore, multiple parameters of preparation and extraction were carried out to determine how to obtain the highest yields of extract.

To meet the predominant objective, the team elected a conventional form of extraction such as the SLE as well as a more unconventional and advanced form of extraction, MAE. Both of these methods were chosen because they generally require the use of less solvent and equipment to obtain an extract, with the exception of the microwave apparatus. Studies from Pap *et al.,* (2013) and Proestos and Komaitis (2008) document that the MAE method can result in less solvent usage and a shorter extraction time which would be beneficial for the commercial markets [94,95]. First, preliminary work was done on the crowberries samples to find the most suitable methods for extraction. The preparation of the crowberries was assessed to determine which preparation would be more efficient by examining two methods: using frozen whole crowberries that were homogenized and lyophilisation of the frozen crowberries. It was clear that lyophilisation

may not be the best choice because the crowberries have a water content of almost 90% and after over 72 hours in the freeze dryer, the sample was not the proper consistency. Also, the lyophilisation process would require the use of the freeze dryer which may be difficult to obtain and transport to the Basse-Côte-Nord as well as the maintenance. Therefore, it seems that it was more efficient and suitable for the region to use frozen homogenized samples. We also decided to assess which solvent treatments of the homogenized samples would be prioritized to obtain the highest yields of phenolic compounds while keeping in mind that the solvent would have to be cosmetic-friendly for the commercial market. Preliminary tests revealed that solvents with a higher dielectric constant or higher polarity allowed for the highest yields which agreed with published studies where ethanol and methanol were the main solvents chosen for extraction [67,91,93. However, for the cosmeceutical and nutraceutical industry, it is generally preferred to not use these types of solvents if possible because the industry is trying to move away from synthesized chemicals into more natural and green solvents. Thus, ethanol was chosen as a standard for comparison as well as water and 1% citric acid were also chosen as they are considered more cosmetic-friendly. These solvents have the advantage of being less harmful to the environmental and humans and are relatively inexpensive.

Next, to determine the most optimal conditions to obtain the highest yields of phenolic compounds, multiple parameters of time, temperature and solvent concentration were examined for both extraction methods. To ensure a wide spectrum of parameters, extractions were performed at one, two and five hours using solvent concentrations of 25:1 to 75:1 ml/g (25-75% for ethanol) at 25°C (for SLE only) and 70°C. Determining the most optimal extraction conditions is important because firstly we can get an idea of the highest yields of phenolic compounds that are present in the crowberries from the region. Also, determining the optimal conditions of temperature and solvent concentration will allow for the appropriate assessment of the equipment and materials needed for efficient extraction on the Basse-Côte-Nord.

Thus, it was then possible to determine that there indeed phenolic content in the crowberries from the Basse-Côte-Nord region and it is comparable to that of other fruits [67,79]. Firstly, for the SLE method, it was determined that a longer extraction time seem to produce higher yields of phenolic compounds (Table 1 and 2, Chapter II). In terms of the ideal solvent concentration, it seemed that a higher concentration of the ethanol mixture extracted the highest yields which is depicted in Figures 1 through 3c of Chapter II. This study on this extraction method in particular corroborates other studies where ethanol is used as a primary extraction solvent as well as the longer extraction time, as many studies examining plant extractions have an extraction time of one hour or more or they use more complex equipment such as high-speed centrifuges. [61,80,93,98]. Temperature seemed to have a very slight effect on the polyphenol yields (Figure 3d, Chapter II) where the increased temperature slightly increased the phenolic compound yields. This is an important conclusion because implies that the extractions can be done at room temperature rather than using a heat source for the extraction and therefore saving time and extra costs of energy and equipment. Secondly, for the MAE method, the results seem to indicate that this method uses significantly less extraction time than the SLE method (Table 1 and 2, Chapter II) where the best extraction time was determined to be 5 minutes for MAE versus 2-5 hours for the SLE method. As with the SLE method, ethanol solvent again seemed to produce the highest yields, however a lower concentration of solvents did not always produce the highest amount of polyphenol. As an example, in the case of antioxidant activity analysis (Figure 6a-c), a higher concentration of ethanol was used to get the highest yields of phenolic compounds, however, the lower concentrations of ethanol were still in a comparable range. Unfortunately, the microwave apparatus at UQTR was unable to produce an accurate result using a lower temperature such as room temperature due to the setup and missing components of the apparatus. The MAE method did not seem to lower the amounts of solvent in all cases as opposed to studies of Li *et al.* (2017) and Pap *et al.* (2012), however it greatly reduced the extraction time which is in corroboration with these studies [89,95].

3.2 Limitations and Outlooks

3.2.1 Methodological limitations

It is important to acknowledge that this study has some relevant limitations surrounding its methods and theories that are critical to future work. Identifying these limitations and possible solutions, could further navigate prospective work on crowberries, or wildberries in general, especially in this type of remote location.

In regard to the methods of this study, the MAE method used at UQTR was unable to do an extraction at a lower temperature without losing the radiation power of the microwave. Some studies choose not to control the temperature, but rather set the power to a maximum wattage [94,95]. However, since it is known from previous studies that phenolic compounds can be sensitive to heat, it was decided to control the temperature versus the power [83,85]. However, it is impossible to know if this had an effect on the result. The microwave apparatus in some studies are able to control pressure studies explain their microwave apparatus to have a component that controls pressure inside the apparatus. [83,85,94,95]. This additional component of controlling the pressure may be better able to control the temperature and power simultaneously and allow a more representative result. It may be possible to create a microwave apparatus using a standard kitchen microwave where the pressure control component could be added. Moreover, there are always upcoming methods arising using new technology such as ultrasonic-assisted extraction which could provide higher yields. Also, all of the analyses were assays which are based on spectrophotometric results. The spectrophotometer measures the concentration of solutes in a solution by measuring the amount of light that is absorbed by the solution and since some phenolic compounds can be sensitive to light and degrade when in the presence of UV rays, it is possible there is a fluctuation if proper care is not taken to make sure the extracts are protected from light at all times before their absorbance measurements are read [97,98]. Therefore, it would be useful to attempt other forms of analyses that do not require the use of the spectrophotometer to determine if correlating results can be obtained to ensure light does not have a significant effect on the phenolic compounds in the crowberry. Some other methods that could be used to quantify

compounds in colorimetric assays are HPLC or LC-MS, however these apparatuses are larger and require more equipment which may not be suitable for the Basse-Côte-Nord at the present time.

Another methodological limitation is that the methods had to be chosen and adjusted according to limitations of the regions. The methods that were chosen for this study, were chosen while keeping in consideration that there is limited access to equipment and materials in the Basse-Côte-Nord region such as the example of choosing homogenization of the frozen crowberries over lyophilisation. Many studies prefer the use of lyophilised materials for the extraction and therefore, it is likely possible that when having the convenience of being able to use more advanced equipment, solvents and methods, the yields could increase. However, one of the objectives of this particular study was to study the phenolic compounds of the crowberry keeping in mind the issues of the region and therefore minimal use of advanced or large equipment.

Finally, one of the objectives of the project was to analyze at the skin and seed residues from the region to see if they were comparable to the analysis of the whole crowberries samples. However, due to time constraints, the residues were unable to be analyzed using the same parameters are the whole crowberry samples. The preliminary moisture content analysis of the residues revealed they had 56% less water content which may indeed produce different results than the whole crowberry. Moreover, the processing facility in which the residues were being obtained from, has now begun a transition into creating cosmetic products and therefore will be discontinuing the food processing and thus the residues.

3.2.2 Theoretical limitations of the Basse-Côte-Nord

The theory of obtaining the crowberries and producing the extracts on site in the region has many limitations. Firstly, obtaining the crowberry samples from season to season may prove to be a challenge in keeping up with number of extracts required for the products for the cosmeceutical and nutraceutical companies. An example of this

limitation, is due to the region in which this project is based on, the team was interested in looking at crowberries from different sites such as mainland sites (landlocked sites) and island sites (sites surround by ocean water) as it is locally known that larger crowberries grow on islands. Therefore, we were interested in obtaining crowberry samples from various sites and testing them independently to see if the crowberries on the islands had a different composition, as the islands had are open to the elements of wind and "salt water spray" from the ocean. However, the season that the crowberry samples were obtained was an unfortunate year for the crowberries and they were scarce; only a small amount could be found on the islands and this intention could not be achieved. This is an example that it is not possible to control the growth of crowberries or their location and therefore impossible to determine the amount that can be acquired year to year. A possible solution for future study is to determine if these types of berries can be grown through agriculture. In Finland, the crowberry is already grown through agriculture and used for wines [2]. This would help ensure the quality and a general quantity of crowberries; however, although it is done in Europe, it will be a long and demanding task as agriculture in sub-arctic regions is a new concept to this region of Québec, especially the Basse-Côte-Nord region, and substantial work will have to be done over time to determine if this could be a solution.

Finally, a future study that could benefit the region would be to study and quantify the skin and seed residues to determine if they have the same or similar quality to the whole crowberry samples. If it is the case, which according to Biopterre (2015), they are quantifiably similar, the crowberry skins and seeds could create another type of extract [99,100]. An example of this would be to examine the oil from the seeds as preceded in a study by Yang and Kortesniemi (2015), suggesting that seed have *Vaccinium* and *Rubus* species linolenic acids have had positive effects on cardiovascular health [101]. Therefore, another path of revenue could potentially include the use of the seeds.

To further validate the use of crowberries in cosmetics as a highly powerful antioxidant component, so further research will have to be done. Firstly, it is important to further quantify and purify the phenolic compounds in the crowberry to realize its full

potential. More research could be done to determine how the phenolic compounds could be mixed with cosmetic materials such as glycerin and determine how well the antioxidants potency holds over time by checking processes such as shelf life and microbial analyses. This research along determining if the crowberry can be grown through agriculture would truly actuate if this the crowberry could be grown and used in cosmetics sustainably.

3.3 Conclusion

To conclude, all the results obtained in this study, indeed demonstrate that there is polyphenolic content, especially in the form of antioxidants activity present in the crowberries from the Basse-Côte-Nord. The results suggest that the yields found in the crowberry are comparable with other fruits and berries already used in the commercial markets. The work has also shown that it is possible to obtain high-yield extracts using ethanol as an extract solvent, however ethanol is not ideal for the cosmetic industry. Thus, water or citric acid would be better suited as the water and citric acid extracts did detect a notable antioxidant activity, however the yields were significantly less for phenolic compounds. The MAE method remarkably reduced the extraction time in some cases, the amount of solvent as well while obtaining higher yields than the SLE method. These results add to the rare studies that are done with crowberry phenolic compounds in Canada, using the MAE method as well as keeping in mind the remoteness of the region. It is certain since these berries are only found in northern climates, that many northern communities who have access to the crowberry, will face many of the complex issues previously mentioned. And therefore, using techniques and protocols that are suitable for the region, it is possible to obtain extracts that are favorable for use in the cosmeceutical and nutraceutical industry while at the same time open the door to further examining the crowberry in these types of regions with hopefulness to aid in economic diversity.

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