Compounds from A. Platanoides Bark, V. Corymbosum Roots & Topical Formulations using Maple Syrup

Raed Omar
University of Rhode Island, mjeo23@aol.com

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COMPOUNDS FROM *A. PLATANOIDES BARK, V. CORYMBOSUM ROOTS & TOPICAL FORMULATIONS USING MAPLE SYRUP

BY

RAED OMAR

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACEUTICAL SCIENCES

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OF

RAED OMAR

APPROVED:

Thesis Committee:

Major Professor

Navindra Seeram
David Worthen
Lori Ciccomascolo
Nasser H. Zawia

DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND
2013
ABSTRACT

The United States is the world’s largest producer of blueberries and the world’s second largest producer of maple syrup. Maine is the nation’s leading producer of wild blueberries, harvesting 82.6 million pounds of wild blueberries. Similarly, the New England region represents roughly 75% of the total U.S. production of maple syrup. These plants play a vital role in New England’s economy and are commonly consumed; however, these plants benefit more than the economy. The secondary metabolites of these plants are both antioxidants, and also anti-inflammatory, antibacterial and anti-viral agents. Blueberries are commonly consumed and have been investigated exhaustively. On the other hand, the roots and stems of the blueberry bush have not been investigated for their phytochemicals. Similarly, our laboratory has investigated two of the most common species tapped for their sap of the maple (Acer) genus. Another species of the maple family that produces sap is the Norway maple tree (Acer platanoides). Investigating the phytochemicals from these plants can revolutionize many industries.
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This dissertation is written in manuscript format.
TABLE OF CONTENTS

ABSTRACT .............................................................................................................................................. ii
ACKNOWLEDGEMENTS ................................................................................................................... iii
PREFACE ................................................................................................................................................ v
TABLE OF CONTENTS .......................................................................................................................... vi
LIST OF TABLES .................................................................................................................................. x
LIST OF FIGURES ............................................................................................................................... xi

PART I. INTRODUCTION

Introduction .......................................................................................................................................... 1

Acer saccharum: Sugar Maple ........................................................................................................... 2

Acer rubrum: Red Maple ................................................................................................................... 2

Acer platanoides: Norway Maple ..................................................................................................... 3

Isolates from Acer genera .................................................................................................................. 3

Maple Syrup ....................................................................................................................................... 9

Vaccinium genus .................................................................................................................................. 11

Vaccinium corymbosum: Highbush Blueberry ................................................................................ 12

Phytochemicals Isolated from the Highbush Blueberry Fruit ....................................................... 13

Biological Activity of the Highbush Blueberry .............................................................................. 14

Antioxidant Activity of the Highbush Blueberry ........................................................................... 15

Anticancer Activity of the Highbush Blueberry ............................................................................. 16

α-Glucosidase Inhibitory Activity of the Highbush Blueberry ...................................................... 17

Shikimate Pathway .......................................................................................................................... 18

Formulations ..................................................................................................................................... 20
Vitamins in Wound Healing ................................................................. 21
Vitamins Assisting with Proper Wound Closure .............................. 21
Role of Sugar in Wound Healing ....................................................... 22
Role of Phenolic Compounds in Wound Healing ......................... 23
References .......................................................................................... 26

PART II. MANUSCRIPTS

First Isolation of Aceraldehyde from Norway Maple (Acer platanoides) Bark ...... 31
Abstract ............................................................................................ 32
Introduction ....................................................................................... 33
General Experimental Procedures .................................................... 35
Source of Plant Materials ................................................................. 36
Extraction and Isolation ................................................................. 35
Analytical High Performance Liquid Chromatography (HPLC-UV) .......... 38
α-Glucosidase Inhibitory Assay ......................................................... 39
Identification of Compounds from Norway Maple Bark ....................... 40
Epi-catechin Aldehyde (Aceraldehyde) ............................................ 40
Results and Discussion ................................................................... 44
References .......................................................................................... 47

A-type Proanthocyanidins Cinnamtannins B1 and D1 Isolated from Highbush
Blueberry (Vaccinium corymbosum) Roots ............................................ 51
Abstract ............................................................................................ 52
Cinnamtannin B1: $^1$H-NMR: ................................................................. 82
Cinnamtannin B1: $^{13}$C-NMR ............................................................... 83
Cinnamtannin D1: $^1$H-NMR .................................................................. 84
Cinnamtannin D1: $^{13}$C-NMR ............................................................... 85
# LIST OF TABLES

| TABLE | PAGE |
|-------|------|
| Table A: Compounds Isolated from Sugar Maple Bark | 4 |
| Table B: Compounds Isolated from Red Maple Stems | 5 |
| Table C: Compounds Isolated from Red Maple Bark | 6 |
| Table D: Compounds Isolated from Ussuri Maple Stems | 7 |
| Table E: Compounds Isolated from Nikko Maple Bark | 8 |
| Table F: Compounds Isolated from Norway Maple Leaves | 9 |
| Table G: Compounds Isolated from Maple Syrup | 11 |
| Table H: Concentration of Phenolic Compounds in Blueberries | 14 |
| Table I: Compounds Isolated from Blueberry Flowers | 15 |
| Table J: $\delta^{1}H$ and $^{13}C$ Data for Cinnamtannin B1 | 60 |
| Table K: $\delta^{1}H$ and $^{13}C$ Data for Cinnamtannin D1 | 61 |
LIST OF FIGURES

| FIGURE | PAGE |
|--------|------|
| Figure 1: Shikimate Pathway: Formation of Benzoic Acids | 19 |
| Figure 2: Shikimate Pathway: Formation of Coumarins | 19 |
| Figure 3: Norway Maple Isolation Scheme | 38 |
| Figure 4: Structures of Chemical Constituents Identified in Norway Maple Bark | 40 |
| Figure 5: Structure of Epi-catechin Aldehyde, Aceraldehyde | 42 |
| Figure 6: Key HMBC Correlations of Epi-catechin Aldehyde, Aceraldehyde | 42 |
| Figure 7: Proposed biosynthesis of Epi-catechin aldehyde, Aceraldehyde | 43 |
| Figure 8: Highbush Blueberry Isolation Scheme | 54 |
| Figure 9: Compounds Isolated from Highbush Blueberry Roots | 58 |
| Figure 10: Cinnamtannins B1 and D1 | 59 |
| Figure 11: Key HMBC Correlations (H → C) of Cinnamtannin B1 | 66 |
| Figure 12: pH of Formulations | 73 |
INTRODUCTION

Approximately 300,000 higher plant species have been identified and only 3–5% have been evaluated for their chemical constituents. Each plant species contain hundreds of organic compounds but many await phytochemical investigation. Thus, there are a very large number of secondary metabolites yet to be discovered.\(^1\)

Living organisms produce organic compounds which can be broken down into primary metabolites and secondary metabolites. Primary metabolites, which include proteins, carbohydrates, fats, and vitamins, are compounds needed by an organism to exist. Secondary metabolites are compounds that are not directly involved in the normal growth, development or reproduction of organisms.

The term “phytochemical” refers to a wide variety of compounds in plants. Phytochemicals are found in plant-based foods such as fruits, vegetables, beans and grains. Thousands of phytochemicals have been studied closely for their beneficial effects on human health.

Due to its global consumption, maple syrup has attracted scientific interest for a better understanding of its benefits beyond its sweet taste. Currently the sugar maple and red maple are the primary trees tapped for their sap which is boiled to produce syrup. However, as sugar maple (*Acer saccharum*) and red maple (*Acer rubrum*) have suffered in the northeastern United States due to an increase in climatic temperature, researchers have sought alternative sources of sap-producing trees. One such tree of interest is the Norway maple (*Acer platanoides*), known for its tolerance to higher temperatures and its ability to produce sap that can be concentrated to syrup.
Our laboratory recently has become interested in investigating the secondary metabolites of maple syrup and other plant parts of the most commonly tapped trees. Our investigation of the phytochemicals of the sugar maple bark and the red maple stems and bark resulted in the isolation and identification of 15 novel compounds. These compounds, mapлексins, rubrumosides and saccharumosides, had potent biological activity, thus prompting us to investigate other hardwoods of sap-producing maple trees.

*Acer saccharum: Sugar Maple*

Sugar maple is the most abundant of the maple species found in New England. Its economic importance in the production of maple syrup is one of the reasons why it is deemed the State Tree of New York. The sugar maple is easy to identify by the clear sap in the petiole.

As previously mentioned, the bark of the sugar maple was investigated for its phytochemicals, resulting in the isolation of four novel phenolic glycosides named saccharumosides. These saccharumosides had cytotoxic activity against human colon tumor cell lines (HCT-116 and Caco02); in contrast, they were non-cytotoxic against non-tumorigenic cell lines (CCD-8Co).

*Acer rubrum: Red Maple*

Similar to the sugar maple, the red maple is tapped for its sap, which is then concentrated into syrup. The State Tree of Rhode Island, the red maple is native to eastern North America and adapts to most soil types and site conditions.

Our investigation of the secondary metabolites of the red maple’s bark and stems resulted in the isolation and identification of nine novel gallotannins named...
maplexins. These gallotannins had potent \( \alpha \)-glucosidase inhibition. Additionally, this study also suggested that the position of the galloyl groups on the glucitol core correlated to the inhibition of \( \alpha \)-glucosidase. Moreover, the three galloylated derivatives attached to different positions of the glucitol moiety were 10–20 fold more potent \( \alpha \)-glucosidase inhibitors than the clinical drug, Acarbose.\(^3\)

**Acer platanoides**: Norway Maple

Norway maple trees are native to Tromsø, Norway and have also been cultivated in Western Europe and in North America.\(^6\) It is an invasive plant grown as a street tree and shade tree and favored due to its tolerance of poor, compacted soils and urban pollution, the Norway maple is deciduous and can reach a height of 30 meters, with a trunk up to 1.5 meters in diameter.\(^7\) The Norway maple tree can live up to 250 years and is commonly confused with the sugar maple tree.

Due to the isolation and identification of novel bioactive compounds from edible and nonedible parts of the *Acer* species, our laboratory was interested in investigating other species in hopes of finding additional novel compounds.

**Isolates from *Acer* genera:**

Maple syrup is the largest commercially available and consumed natural product that is obtained entirely from the sap of deciduous trees. The sugar maple tree is the most commonly tapped for its sap. Although the sap is not as sweet as that of the sugar maple, the red maple and Norway maple are trees that also produce sap. The popular consumption of maple syrup has generated interest in isolation and characterization of its phytochemical constituents. Our laboratory has investigated
sugar maple bark and red maple stems and bark. *In vitro* studies suggest that the consumption of these isolated phytochemicals may have health benefits.

Fifteen phenolics were isolated from the bark of the sugar maple tree (See Table A). Four of these compounds were novel phenolic glycosides shown to be cytotoxic against human colon cancer cells while non-cytotoxic against non-tumorigenic cell lines. However, more studies would need to be conducted as *in vitro* activity does not correlate to *in vivo* effects.²

**Table A: Compounds Isolated from Sugar Maple Bark**², ⁸

| Compounds Isolated from Sugar Maple Bark                      |
|--------------------------------------------------------------|
| koaburside                                                  |
| vanilloloside                                                |
| icariside E₄                                                 |
| scopoletin                                                  |
| saccharumosides A                                            |
| saccharumosides B                                            |
| saccharumosides C                                            |
| saccharumosides D                                            |
| cleomiscosin C                                              |
| cleomiscosin D                                              |
| 5′-demethylaquillochin                                      |
| syringaresinol-β-D-glucopyranoside                           |
| 3,5-dimethoxy-4-hydroxybenzyl alcohol 4-O-β-D-glucopyranoside|
| 3,4,5-trimethoxyphenyl 1- O-β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside|
| 4-hydroxymethyl-2-methoxyphenyl 1- O-β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside|

Thirteen gallic acid derivatives were isolated from the stems of the red maple tree (See Table B). Of those thirteen phenolics, five novel gallotannins named maplexins with very potent α-glucosidase activity were isolated. Gallotannins are gallic acid derivatives that are esterified to a carbohydrate core. The galloyl groups attached to the glucitol varied in number and location. This study suggests the number
and position of galloyl groups and location are directly correlated to the biological activity of these compounds. Ginnalin A has been investigated for its cytotoxic effects and determined to be more effective than both B and C against human colon tumorigenic cells (HCT-116) and breast cancer cells (MCF-7). This evidence further compels the investigation of other Acer species for their bioactive compounds.

**Table B: Compounds Isolated from Red Maple Stems**

| Compounds Isolated from Red Maple Stems |
|----------------------------------------|
| ginnalin A                            |
| ginnalin B                            |
| ginnalin C                            |
| maplexin A                            |
| maplexin B                            |
| maplexin C                            |
| maplexin D                            |
| maplexin E                            |
| gallic acid                           |
| methyl gallate                        |
| methyl syringate                      |
| 3,6-di-O-galloyl-1,5-anhydro-D-glucitol|
| 3,4-dihydroxy-5-methoxybenzoic acid methyl ester |

Our investigation of the red maple stems prompted further investigation into the bark of the tree. This study resulted in the identification of six novel compounds and 11 known phenolics (See Table C). The three galloylated derivatives attached to positions 1,5-anhydro-glucitol were 10–20 fold more potent α-glucosidase inhibitors than the clinical drug, Acarbose. This suggests the location and number of galloyl groups is correlated to the biological activity for the inhibition of α-glucosidase.
### Table C: Compounds Isolated from Red Maple Bark

| Compounds Isolated from Red Maple Bark |
|----------------------------------------|
| maplexin A                             |
| maplexin B                             |
| maplexin C                             |
| maplexin D                             |
| maplexin E                             |
| maplexin F                             |
| maplexin G                             |
| maplexin H                             |
| maplexin I                             |
| rubrumoside A                          |
| rubrumoside B                          |
| nymphaeoside A                         |
| gallic acid                            |
| ginnalin A                             |
| ginnalin B                             |
| ginnalin C                             |
| methyl vanillate                        |

Hardwoods from other *Acer* species have also been investigated for their phytochemicals. The stems of the Ussuri maple (*Acer barbinerve*) were investigated and resulted in the isolation and characterization of 13 compounds (See Table D).\(^9\)

Furthermore, the compounds isolated are ubiquitous and commonly found in higher plants.\(^{10-12}\) Among these, methyl gallate and gallic acid were also previously isolated from red maple bark and stems.\(^3,4\)
Table D: Compounds Isolated from Ussuri Maple Stems

| Compounds Isolated from Ussuri Maple Stems |
|------------------------------------------|
| methyl gallate                           |
| methyl gallate-4-O-β-D-glucoside         |
| methyl gallate-3-O-β-D-glucoside         |
| gallic acid                              |
| protocatechic acid                       |
| vanillic acid                            |
| p-tyrosol                                |
| (+)- catechin                            |
| (-)- epi-catechin                        |
| (-)-epi-catechin-3-O-gallate             |
| hirsutrin                                |
| hyperin                                  |
| quercitrin                               |

Another Acer species investigated for its phytochemicals was the Nikko maple (Acer nikoense). The Nikko maple bark was investigated because it was used in traditional Japanese folk medicine for hepatic disorders. The bark has also been investigated for anticancer, anti-inflammatory, antifungal and antibacterial effects. Two novel compounds, acerogenin A and acerogenin B, along with nine known phenolic compounds were isolated and characterized (See Table E). These were evaluated for their role as inhibitors of Na+-glucose cotransporter. Na+-glucose cotransporter is a protein membrane that plays an important role in the reabsorption of glucose in the kidneys. Additionally, the compounds isolated from the Nikko maple were evaluated for their anti-inflammatory effects. All compounds, with the exception of aceroside I, inhibited inflammation. Acerogenin A and acerogenin B moderately inhibited SGLT1. SGLT1 is expressed in the small intestine and responsible for the reabsorption of glucose. It is believed the inhibition of SGLT could decrease the
reabsorption of glucose resulting in an increase in urinary sugar excretion which may benefit type 2 diabetes patients.

The biological activity of these compounds from the different *Acer* species suggests investigating other species for novel phytochemicals that may have health benefits.

**Table E: Compounds Isolated from Nikko Maple Bark**\(^\text{13}\)

| Compounds Isolated from Nikko Maple Bark |
|-----------------------------------------|
| acerogenin A                            |
| acerogenin B                            |
| acerogenin D                            |
| acerogenin K                            |
| acerogenin M                            |
| aceroside I                            |
| aceroside II                           |
| aceroside IV                           |
| (-)-centrolobol                        |
| (+)-rhododendrol                       |
| (+)-catechin                           |

The leaves of the Norway maple were previously investigated but only resulted in the identification of three compounds (See Table F).\(^\text{14}\) One of the three compounds, cyanidin 3-(2”,3”-digalloyl-β-glucopyranoside), was novel while the other two, cyanidin 3-(2”-galloyl-β-glucopyranoside) and cyanidin 3-β-glucopyranoside, have been previously reported. Thus investigating the bark of the Norway maple tree is essential since it is possible that these compounds can be re-isolated as well as novel compounds may be obtained.\(^\text{14}\)
Table F: Compounds Isolated from Norway Maple Leaves

| Compounds Isolated from Norway Maple Leaves |
|--------------------------------------------|
| cyanidin 3-β-glucopyranoside               |
| 3-(2”-galloyl-β-glucopyranoside)           |
| cyanidin 3-(2”-3”-digalloyl-β-glucopyranoside) |

Our laboratory has investigated several different species from the *Acer* genus.\(^2\)\(^-\)\(^4\)\(^-\)\(^15\)-\(^17\) The barks of many trees have been investigated, resulting in the isolation and characterization of many novel compounds. One of the most well-known anticancer compounds, taxol, was isolated from the bark of the Pacific yew tree. Taxol is one example of many phytochemicals that are used in medicine; its benefits establish a firm basis to investigate all parts of plants rather than only their edible parts.

Understanding the differences between compounds from different species of the *Acer* genus may yield insight into the chemotaxonomic distribution of compounds. Additionally, it could explain which compounds are organ-specific since research has indicated that plants commonly contain organ-specific distributions of secondary metabolites.\(^18\) Therefore, phytochemical isolation efforts on various plant parts have potential for the discovery of novel bioactive compounds.

**Maple Syrup:**

The province of Quebec, Canada is the largest producer of maple syrup and is responsible for about three-quarters of the world’s output. Vermont is the largest producer in the United States, producing approximately 5% of the world’s maple syrup.

Maple syrup is graded based on its viscosity and color. Sucrose is the most prevalent sugar in maple syrup accounting for over 61% of maple syrup’s makeup. To
produce maple syrup, sap must first be collected and boiled down 40x to obtain pure syrup.

Understanding the phytochemicals of maple syrup may lead to knowledge of additional benefits beyond its sweet taste. Our laboratory has extensively investigated maple syrup for its constituents, and evaluated its constituents for their biological activity. This investigation resulted in the identification of more than 70 compounds, some of which have potent antioxidant activity (See Table G).\textsuperscript{15-17} Maple syrup’s constituents, including high sugar content and a rich polyphenolic makeup, make it ideal for a topical formulation. The anti-inflammatory activity of the polyphenols and the high-sugar concentration could work synergistically as an anti-aging and moisturizing formulation.
Table G: Compounds Isolated from Maple Syrup

| Compounds Isolated from Maple Syrup | |
|------------------------------------|------------------------------------------|
| (E)-3,3′-dimethoxy-4,4′-dihydroxy stilbene | gentisic acid |
| 1-(2,3,4-trihydroxy-5-methylphenyl)-ethanone | guaiacylglycerol-β-coniferyl ether |
| 1-O-galloyl-β-D-glucose | homovanillic acid |
| 2-Hydroxy-3′,4′-dihydroxyacetophenone | isoquercetin |
| 2,4,5-Trihydroxyacetophenone | kaempferol 3-O-galactoside |
| 3′,4′,5′-Trihydroxyacetophenone | lyoniresinol |
| 4-methoxycinnamic acid | methyl gallate trimethyl ether |
| 5-methoxy-trans-dihydrodehydrodiconiferyl alcohol | p-coumaric acid |
| astragalin | potocatechuic acid |
| benzoic acid | quebecol |
| C-veratroylglycerol | quereitrin |
| catechaldehyde | resorcylic acid |
| catechin | rutin |
| catechol | scopoletin |
| chlorogenic acid | secoisolariciresinol |
| coniferyl alcohol | sinapic acid |
| dihydroconiferyl alcohol | syringaldehyde |
| dihydrodehydrodiconiferyl alcohol | syringenin |
| epi-catechin | syringic acid |
| ferulic acid | syringol |
| fraxetin | syringoyl methyl ketone |
| gallic acid | vanillic acid |
| guaiacylglycerol-β-O-4′-dihydroconiferyl alcohol | vanillin |

**Vaccinium genus:**

*Vaccinium* is a genus of shrubs in the Ericaceae family. The fruit of many different species are available commercially and widely consumed. These berries include the cranberry, blueberry, bilberry, whortleberry, lingonberry, cowberry and huckleberry. The genus contains about 450 species and lives in habitats such as bogs and acidic woodlands. The plant structure differs between species as some lay low to the ground such as dwarf shrubs and some are larger shrubs growing up to 12-feet tall. The fruits are usually berries and are red or bluish with purple juice.
**Vaccinium corymbosum: Highbush Blueberry**

A bush native to the United States, the highbush blueberry can grow up to 12-feet tall.19 The highbush blueberry gets its name from its tall stature compared to the low-lying, lowbush blueberry. Its twigs are yellow-green most of the year, but turn dark red in winter months. Its leaves are deciduous, alternate, simple, elliptic or ovate and slightly waxy on top. The white or pink flowers are small and urn-shaped with five petals and each cluster contains approximately eight to ten flowers. Flowering of the highbush blueberry occurs from February to June, and fruiting occurs from April to October.19

The highbush blueberry is widespread in eastern North America. The most common native habitat is found in moist or wet soil of moderate to high acidity such as in marshes, swamps and lakes. Nonetheless, the bush also can be found in dry areas, such as dunes, barrier beaches and rocky hillsides.19

The highbush blueberry produces fruit every year and is self-fertile. Cross-pollination increases fruit set and results in larger and earlier berries with more seeds. Bees are the primary pollinators. However, the seeds may be dispersed by birds and mammals.19 In the southern portion of its range, highbush blueberry seeds have thick seed coats and require cold stratification before germination. Those from northern regions produce thinner seed coats and germinate in the autumn after dispersal. Plants have also been noted to produce root sprouts which emerge up to two meters away from the parent plant.20
Phytochemicals Isolated from Highbush Blueberry Fruit:

Small berries constitute one of the important sources of potential health-promoting phytochemicals.\textsuperscript{21,22} These fruits are rich sources of phenolic compounds such as phenolic acids, anthocyanins, proanthocyanidins and other flavonoids, which have been investigated for their potential health-promoting effects.\textsuperscript{21,22} The content of phenolics in berries is affected by the degree of maturity at harvest, cultivar, environmental conditions, storage conditions and processing. Polyphenols, abundant in blueberries, have been seen to produce favorable nootropic, antioxidant and anti-inflammatory effects.\textsuperscript{20-22}

Many \textit{in vitro} and \textit{in vivo} studies suggest that the flavonoid-rich fruits of the blueberries (\textit{Vaccinium} spp.) have the ability to inhibit and prevent diseases. The fruits contain a variety of phytochemicals, including anthocyanins, flavonols, proanthocyanidins, stilbenes and triterpenes. These phytochemicals could contribute to human health benefits. Blueberry constituents are antioxidants that counter oxidative stress and decrease inflammation.

Due to their superior antioxidant potential, the anthocyanins found in blueberries may play a major role in the inhibition of oxidative processes linked to diseases and cancer. Blueberries are rich in anthocyanins, including petunidin, malvidin, delphinidin, peonidin and cyanidin.\textsuperscript{23} Understanding the phytochemical constituents of these plants may help in the elucidation of their health benefits. Moreover, secondary metabolites are known to be organ specific, as parts of the plant produce different secondary metabolites. This organ-specificity justifies the need to investigate both edible and non-edible parts of plants. Although non-edible plant parts
are not consumed, secondary metabolites can be isolated from these organs and used in dietary supplements.

As previously mentioned, investigating the phytochemicals of different plants and plant parts provides a better understanding of the plants’ benefits to human health. Zheng et al have isolated and quantified the major secondary metabolites found within the blueberry fruit (See Table H). Additionally, Wang et al isolated gallic acid, syringic acid, protocatechuic acid, β-sitosterol, ursolic acid and β-sitosterol-β-D-glucoside. Other polyphenols identified include flavonol glycosides and catechins.

Table H: Concentration of Individual Phenolic Compounds in Blueberries

| Compounds                  | % in Highbush Blueberry |
|----------------------------|-------------------------|
| chlorogenic acid           | 29                      |
| delphinidin 3-galactoside  | 8.5                     |
| malvidin 3-galactoside     | 6.8                     |
| petunidin 3-galactoside    | 6.5                     |
| malvidin 3-arabinoside     | 5.8                     |
| petunidin 3-arabinosde     | 5.8                     |
| malvidin 3-glucoside       | 5.2                     |
| delphinidin 3-glucoside    | 5.1                     |
| quercetin 3-galactoside    | 4.8                     |
| delphinidin 3-arabinoside  | 4.3                     |
| myricetin 3-arabinoside    | 3.9                     |
| quercetin 3-arabinoside    | 3.9                     |
| petunidin 3-glucoside      | 3.2                     |
| quercetin derivative       | 2.9                     |
| quercetin 3-glucoside      | 1.3                     |
| kaempferol 3-glucoside     | 0.2                     |
| kaempferol derivative      | 0.2                     |

Biological Activity of the Highbush blueberry:

Blueberries contain a diverse range of phytochemicals which have antioxidant, anticancer and anti-inflammatory biological properties. The major
classes of berry phenolics are anthocyanins, flavonols, flavanols, ellagitannins, gallotannins, proanthocyanidins and phenolic acids. These extracts, once purified, are composed of phenolics that may work synergistically and have been investigated in vitro for their biological activities.\textsuperscript{18, 20, 22, 24, 27-29}

Table I: Compounds Isolated from Blueberry Flowers\textsuperscript{18}

| Compounds Isolated from (Vaccinium corymbosum) Blueberry Flowers |
|---------------------------------------------------------------|
| rosin                                                         |
| cinchonain                                                   |
| methyl caffeate                                              |
| 5-O-caffeoylshikimic acid                                    |
| 5-O-caffeoylquinic acid                                       |
| trans-coumaric acid methyl ester                             |
| 5-O-caffeoylquinic acid methyl ester                         |
| 3,5-dicaffeoylquinic acid methyl ester                        |
| 5-O-coumaroylquinic acid                                     |
| 5-O-coumaroylquinic acid methyl ester                        |
| 2'-O-β-D-glucosylrosine                                       |
| quercetin-3-O-β-D-glucoside                                   |
| quercetin-3-O-β-D-galactoside                                  |
| quercetin-3-O-α-L-arabinopyranoside                           |
| myricetin-3-O-β-D-galactoside                                  |
| quercetin-3-O-(6''-O-coumaroyl)-β-D-glucoside                 |
| quercetin-3-O-(2''-O-coumaroyl)-β-D-glucoside                 |
| kaempferol-3-O-(6''-O-coumaroyl)-β-D-glucoside                |
| (4S,8R,9S)-4,8-Bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-  |
| 5,9-dihydroxy-2H,8H-benzo[1,2-b:3,4-b]dipyran-2-one           |
| catechin-[8,7-e]-4b-(3,4-dihydroxy-phenyl)-dihydro-2(3H)-pyranone |

Antioxidant Activity of the Highbush Blueberry:

Antioxidant compounds in food play an important role in preventive health. Evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease.\textsuperscript{18, 20, 22, 24, 27-29} Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Antioxidants like vitamin C,
vitamin E, phenolic acids and phytoestrogens have been recognized as having the potential to decrease the risk of disease. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a variety of physical and chemical properties.

Blueberries are known for their high oxidant radical absorbance capacity (ORAC) activity to reverse age-related deficits in cognitive function. Blueberries, spinach and strawberries were chosen for a study to determine the ability of short-term dietary supplementation to reverse age-related deficits in motor and cognitive functions. Blueberries were the most effective of the three foods to show signs of reversing age-related deficits in behavioral activities of rats.

Moreover, another study conducted at the University of California concluded a diet rich in foods and beverages containing flavonoids may decrease the risk of developing atherosclerosis, due to the ability of these compounds to inhibit low-density lipoprotein (LDL) oxidation and platelet aggregation. Therefore, phytochemicals present in antioxidant-rich foods may be beneficial in reversing the effects of behavioral aging.

**Anticancer Activity of the Highbush Blueberry:**

Berry extracts were evaluated for their ability to inhibit the growth of human oral (KB, CAL-27), breast (MCF-7) and colon (HT-29, HCT116) tumor cell lines at different concentrations. As the concentration of berry extract increased, the inhibition of cell proliferation also increased in all tumorigenic cell lines. This suggests that the phytochemicals work synergistically.
However, independent phytochemicals can be assayed for their biological activity. Ursolic acid isolated from the highbush blueberry has been assayed against several cell lines; and, research suggests that it is a potent cytotoxic agent in human leukemia cells, lymphocytic leukemia cells p-388, human lung carcinoma cell A-549, and human colon (HCT-8) and mammary tumor cells (MCF-7). This further suggests blueberries contain additional benefits beyond their basic nutritional value.

**α-Glucosidase Inhibitory Activity of the Highbush Blueberry:**

Recently the highbush blueberry has been investigated for its inhibition of α-glucosidase. In comparison to Acarbose, all blueberry extracts showed similar α-glucosidase inhibition capabilities. Preliminary studies with the *Vaccinium* genus showed that a proanthocyanidin (PAC) rich fraction had a lower IC$_{50}$ value than an anthocyanin-rich fraction, suggesting a PAC-rich extract is more beneficial for the inhibition of α-glucosidase.

Again, *in vitro* studies have focused on identifying the phytochemical constituents responsible for the observed anticancer and α-glucosidase inhibitory activity of these fruits. The highbush blueberry fruits were shown to inhibit α-amylase and α-glucosidase. Blueberry fruits and flowers have been researched for their phytochemicals; however, the roots have not been investigated. Previously published research supports the need for researching these roots for other possible bioactive compounds that potentially may be used medicinally or in nutraceuticals.

Because secondary metabolites are formed through specific biosynthetic pathways, understanding these pathways is important. One of the most common pathways is the shikimate pathway.
**Shikimate Pathway:**

Investigating biosynthetic pathways is important because investigating the metabolic pathways of natural products allows for a fundamental understanding of the origin of secondary metabolites. There are many different pathways such as the shikimate pathway, mevalonate pathway and the acetate pathway. The shikimate pathway is one of the most common metabolic pathways and is responsible for the formation of phenylpropanoids, coumarins and even phenolic acids (See Figures 1 and 2). The shikimate pathway is found in bacteria, fungi and plants. Phenylalanine and tryptophan are essential amino acids that are consumed while tyrosine is directly derived from phenylalanine. Phenylalanine, tyrosine and tryptophan represent a majority of the precursors for nearly the entire output of aromatic biosynthesis. In higher plants on the other hand, the amino acids are the precursors for many secondary metabolites with aromatic ring structures. Phenylalanine and tyrosine form the basis of the C6-C3 units found in many natural products including coumarins, lignans and flavonoids.
Figure 1: Shikimate pathway: Formation of Benzoic Acids

Figure 2: Shikimate Pathway: Formation of Coumarins
Formulations:

Formulations are developed based on desired purpose for treating conditions such as acne, eczema, burn healing, anti-aging, etc.\textsuperscript{32} The profile will include: the intended therapeutic indication, the preferred dosage form (cream, gel, ointment, etc.), the anticipated product strength (% active ingredient), the desired release profile with skin penetration goals, target shelf life and the desired cosmetic/aesthetic properties.\textsuperscript{32} The formulation must allow for optimal penetration of the active ingredient into the skin. Skin pH is approximately 5.5. Thus the pH of the formulation may change following application to the skin. Many factors contribute to an optimal aesthetic and effective formulation. The aesthetic appeal of the formulation is just as important to the customer as its intended use, and an especially useful tool when attempting to differentiate oneself from one’s competitors.

 Healing a wound is a very complex process with many steps. Anti-aging, moisturizing and even antibacterial formulations need to meet a lot of the same criteria. A wound-healing formulation, on the other hand, must take all of those factors into consideration, and thus would make a great product for other uses.

 In this respect, particular interest has been shown in natural products such as honey, due to its healing properties exhibited \textit{in vitro}.\textsuperscript{33} One study of honey \textit{in vitro} showed extraordinary wound-healing activity. Investigated for its wound-healing properties, honey’s powerful healing abilities have been attributed to its sugar content as a result of hyaluronic acid’s composition of polysaccharides. These chains are made from modifications of the monosaccharide glucose.\textsuperscript{21} Flavonoid-rich plants have been
investigated for their wound-healing ability and are known to expedite wound healing. Thus, high sugar content in conjunction with a high polyphenolic profile may expedite wound healing by reducing inflammation and increasing collagen stimulation.

**Vitamins in Wound Healing:**

Vitamins such as vitamin A (retinol), C (ascorbic acid) and E (α-tocopherol) have been investigated extensively for their beneficial effects in topical applications. Research has found that vitamins C and E can help protect the skin against sun damage. It also has been theorized that they may reverse discoloration and wrinkles associated with aging and overexposure to the sun. These powerful antioxidants stabilize free radicals in order to slow the damage produced by harmful UVA–UVB rays. However, vitamins have never been used in conjunction with maple syrup. Fat-soluble and water-soluble applications have also been of interest due to the capabilities of dermal penetration *in vivo.*

**Vitamins Assisting with Proper Wound Closure:**

Compounds with high antioxidant activity such as vitamin E and vitamin C are commonly used post-surgery for treating scarring and assisting in wound healing. Our laboratory has investigated the phytochemicals isolated from maple syrup that show promising antioxidant activity compared to vitamin C. These phytochemicals’ potent antioxidant activity supports the use of maple syrup for its anti-inflammatory effects and ultimately for topical applications in wound healing.

Moreover, Bartlett *et al*, 1942, have shown that a sufficient depletion of Vitamin C produces a decreased tensile strength in healing skin wounds, even when
supplemented orally and intravenously. Vitamin A (retinol) has proven to be effective when supplemented in wounds as exhibited by Seifter et al. The importance of vitamin concentrations in a formulation extends beyond texture and aesthetic appeal. It is directly related to irritation when applied topically and creates adverse effects including a rash or even more severely a burn. In addition to the irritation caused by high dosing of vitamins, vitamins A, D, E and K are lipid soluble and not water-soluble, thereby increasing the complexity of the dosage form and penetration of each ingredient that is concentration dependent.

Although there are vitamins present in maple syrup, they are in the very small concentrations of approximately 0.33 mg per 100 grams maple syrup for vitamins B2 and B3. Thus, the formulation needs to have vitamins added to those already present. Furthermore, between 7–10% of maple syrup is added to the formulation to maintain optimal consistency and texture when applied. With a low concentration of maple syrup, vitamin concentrations will also be low and therefore will not contain an effective dose.

**Role of Sugar in Wound Healing:**

Sugar content has been shown to increase skin elasticity thereby helping with proper wound closure. Maple syrup’s potent antioxidant activity, high sugar content, and phenolic abundance suggest that, in ways similar to honey, maple syrup can expedite wound-gap closure. Honey and sugar or sugar paste have been used to treat wounds for decades. They are considered to be antimicrobial and have been associated with scarless healing in wounds. Hyaluronic acid consists of disaccharide chains made from modifications of the monosaccharide glucose. It is
theorized that glucose in honey or glucose derived from sugar may be converted into hyaluronic acid at the wound surface, forming an extracellular matrix that promotes wound healing.\textsuperscript{40} Fetal wounds heal without scar formation possibly due to their being rich in hyaluronic acid and their lack of excessive collagen.\textsuperscript{13} Therefore, the glucose in honey or that derived from sugar may facilitate a balance between hyaluronic acid and collagen, similar to that found in fetal wounds.\textsuperscript{40}

Moreover, Cooper \textit{et al} reported that a minimum of 29\% sugar content is required for inhibition of certain bacteria in wounds.\textsuperscript{41} This may establish a basis from which to confirm our hypothesis of sugar being a requirement for optimal wound healing. Furthermore, their findings reported better inhibition at higher concentrations, compared to the lower concentrations of honey. Neutrophils must cleanse the wounded area of foreign particles and bacteria and are then extruded by macrophages for proper wound healing.\textsuperscript{42} Thus, sugar plays a very important role inhibiting bacterial growth, and thus, maple syrup may play a role in beneficial wound healing.

\textbf{Role of Phenolic Compounds in Wound Healing:}

Flavonoids have been shown to exhibit beneficial wound-healing properties as reported by Mukherjee \textit{et al}.\textsuperscript{43} The methanol extract of \textit{Hypericum patulum} leaves were investigated in order to evaluate their wound-healing potential on different experimental models of wounds in rats. The methanol extract of leaves in the form of an ointment with two different concentrations (5\% and 10\% w/w ointment of leaf extract in simple ointment base) was evaluated for wound-healing potential in an excision-wound model and an incision-wound model in rats. Both concentrations of the methanol extract ointment showed significant responses in both wound types.
tested when compared with the control group. They measured these properties in terms of wound contracting ability, wound closure time, regeneration of tissues at wound site and the tensile strength of the wound, which were comparable to those of the standard drug, nitrofurazone ointment. The 10% (w/w) extract ointment treated groups showed significant wound healing from after the fourth day, which was comparable to that of nitrofurazone in comparison to the five percent (w/w) showed significant wound healing from the eighth day.  

Moreover, catechins, a sub-class of polyphenols, have been shown to improve scar healing and wound closure comparable to commercially available topical ointments. Similarly, epi-catechin gallate has been shown to expedite wound healing in vivo. This was a result of an increase in important endothelial growth factors. The expression of endothelial growth factors such as nitric oxide synthase, cyclooxygenase and arginase-I, are vital for maintaining the different phases of wound healing and increasing the number of new blood vessels formed during wound healing.

In addition, phenolic acids, another sub-class of polyphenols, possess powerful free-radical scavenging activity. One study administered caffeic acid to wounded rats for 14 days. Wound tissues showed a significant increase in nitric oxide levels that resulted in the development of the epithelium. The rats treated with caffeic acid showed significant improvement over those left untreated. 

Although several subclasses of polyphenols have been investigated for their wound healing effects, this does not hold true for all compounds. Resveratrol, a stilbene found in abundance in red wine, has been examined for its wound-healing properties. Oral administration of resveratrol significantly delayed wound healing in
mice as measured by the sizes of wounds. The sizes of the wounds measured in the
group supplemented with resveratrol were significantly larger from day 2 and
onward. On the other hand, topical application of grape seed extract has shown to
increase endothelial growth factors resulting in a hyper-proliferative epithelial region
and higher cell density. This has been shown to expedite wound gap closure.
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First Isolation of Aceraldehyde from Norway Maple (Acer platanoides) Bark

Raed Omar, Tao Yuan, Liya Li, Hang Ma, and Navindra P. Seeram*

Bioactive Botanical Research Laboratory, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island 02881, United States

*Corresponding author. Tel.: 401-874-9367, Fax: 401-874-5787, E-mail: nseeram@uri.edu

This manuscript is written and formatted for the Journal of Natural Products.
Abstract: This is the first report of epi-catechin aldehyde, assigned the common name of aceraldehyde, being isolated and characterized from the bark of the Norway maple tree (*Acer platanoides*) along with 19 known phenolic compounds. All compounds were elucidated on the basis of spectroscopic analysis including 1D and 2D NMR data and compared to published NMR when available. These compounds were evaluated for their α-glucosidase inhibitory activity.
**Introduction:** The barks of many trees have been investigated resulting in the isolation and characterization of many novel compounds. One of the most well-known anticancer compounds, taxol, was isolated from the bark of the Pacific yew tree. However, it is not feasible to isolate large quantities of this compound from the bark of this tree, therefore taxol has been synthesized and is now commonly used as a chemotherapeutic agent. This establishes a firm basis to investigate all parts of plants rather than only edible parts.

Moreover, our laboratory has previously investigated the sugar maple’s bark and the red maple’s bark and stems.\(^1\)\(^-\)\(^3\) The phytochemical investigations of the hardwoods of the sugar maple tree and red maple tree resulted in the isolation of: four novel phenolic glycosides from the sugar maple bark; and nine novel gallotannins and two new phenolic glycosides from red maple bark and stems. These novel compounds show promising bioactivity.\(^1\)\(^-\)\(^3\)

Maplexins A–E were isolated from red maple stems and examined for their \(\alpha\)-glucosidase inhibitory activity. The maplexins that contained two galloyl groups were more effective \(\alpha\)-glucosidase inhibitors than those with only one galloyl group. This preliminary study suggested that number and location of galloyl groups correlated with the inhibitory activity of these compounds. Maplexins F, G, H and I, isolated from the red maple bark, were potent \(\alpha\)-glucosidase inhibitors with up to 20-fold more potent inhibition compared to the control, Acarbose.\(^2\) This study then provided additional evidence confirming that the number of galloyl groups and relative
locations of these groups on the glucitol core affect the inhibitory activity of these compounds.

Additionally, crude extracts of red maple tissues such as branches, wood of branches, bark of branches, stem bark and whole twigs were evaluated for their antioxidant capacity. The study, designed to investigate natural antioxidants, indicated the stem bark had the most potent antioxidant capacity. The bark of branches also displayed antioxidant activity, albeit less active than the stem bark.

Saccharumosides are phenolic glycosides isolated from the sugar maple. These phenolics also showed promising biological activity. They were cytotoxic against human colon tumorigenic (HCT-116 and Caco-2) cells while non-cytotoxic against non-tumorigenic cell lines.

Hardwoods from other Acer species have also been investigated for their phytochemicals. The stems of the Ussuri maple (Acer barbinerve) were investigated and resulted in the isolation and characterization of 13 compounds. Furthermore, the compounds isolated are ubiquitous and commonly found in higher plants. Methyl gallate and gallic acid were also previously isolated from red maple bark and stems.

Another Acer species investigated for its phytochemicals was the Nikko maple (Acer nikoense). Two novel compounds, acerogenin A and acerogenin B were isolated and characterized. They were also evaluated for their role as inhibitors of the Na+-glucose cotransporter, which plays an important role in the reabsorption of glucose in the small intestine. Additionally, the compounds isolated from the Nikko Maple were evaluated for their anti-inflammatory effects. Acerogenin A, aceroside B and aceroside IV had the most potent activity.
The Nikko maple bark was investigated because it was used in traditional Japanese folk medicine for hepatic disorders. The bark has also been investigated for its anticancer, anti-inflammatory, antifungal, and antibacterial effects. The biological activity of these compounds isolated from the *Acer* genus invokes investigation of other species from this genus for novel phytochemicals that may have biological properties.

The red maple and sugar maple did not have any compounds in common. However, compounds isolated from the red maple hardwoods including stems and barks contained gallotannins named maplexins A–I and novel phenolic glycosides named rubrumosides A and B. The sugar maple also contained phenolic glycosides named saccharumosides A–D. Previous investigation of these hardwoods has prompted investigation of other barks within the same genus to compare phytochemicals between species belonging to the same genus. One such tree is the Norway maple tree, native to Tromsø, Norway, and cultivated in Western Europe and in North America.\textsuperscript{10}

The Norway maple is grown as a shade tree and favored due to its tolerance of pollution. To date, there is no published literature on the bark of this tree. The investigation of its leaves has resulted in the isolation of three compounds, cyanidin 3-β-glucopyranoside, 3-(2”-galloyl-β-glucopyranoside) and cyanidin 3-(2”,3”-digalloyl-β-glucopyranoside). However, to our knowledge, no phytochemical investigation has been conducted on the hardwood of this species.

**General Experimental Procedures:** Semi-preparative HPLC separations were performed on a Hitachi Elite LaChrom system consisting of an L2130 pump, an L-
2200 auto-sampler, an L-2455 diode array detector and a Phenomenex Luna C18 column (250 × 10 mm, S-5 µm), all operated by EZChrom Elite software. Medium-pressure liquid chromatography (MPLC) separations were carried out on pre-packed C18 columns (4 × 37 cm) connected to a DLC-10/11 isocratic liquid chromatography pump (D-Star Instruments, Manassas, VA, USA) with a fixed wavelength detector. All solvents were of ACS- or HPLC-grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA) through Wilkem Scientific (Pawtucket, RI, USA). Silica gel (230–400 mesh, Sorbent Technologies), Sephadex LH-20 gel (Amersham Biosciences) and MCI gel (CHP20P, 63–150 µM, M & M Industries Inc.) were used for column chromatography; and, pre-coated silica gel GF254 plates (Whatman Ltd., Maidstone, England) were used for TLC analysis.

**Sources of Plant Materials:** The bark of Norway maple was collected in the Fall of 2012 by our laboratory personnel and identified by Mr. J. Peter Morgan (Senior Gardener, College of Pharmacy, University of Rhode Island). A voucher specimen (16JPM2APL51910B) has been deposited in the Heber-Youngken Herbarium and Greenhouse, College of Pharmacy, University of Rhode Island.

**Extraction and Isolation:** The air-dried powder of the bark (4.2 kg) of the Norway maple was extracted by maceration with methanol (5 L × 3 times for 7 days per time period) at room temperature to afford 300 g of crude extract (See figure 3). The extract was suspended in distilled water (1 L) and then extracted successively with ethyl acetate (1 L × 3 times) and n-butanol (1 L × 3 times). The ethyl acetate fraction (100 g) was chromatographed over a column (5 × 50 cm) of MCI gel (MeOH–H2O, 50:50 v/v to 90:10 v/v) to yield three fractions (A–C). Fraction A (47.53 g) was subjected to
silica-gel chromatography (CC) eluted with chloroform-methanol (20:1 v/v to 2:1 v/v) in a gradient, to obtain two fractions (A1−A2). Fraction A1 was separated by semi-preparative HPLC eluted with MeOH−H2O (20:80 v/v to 80:20 v/v in 30 min, 2 mL/min) to yield compounds 1, 2, 5, 12 and 19.

Further purification of A2 was chromatographed over a column (3 × 70 cm) of Sephadex LH-20 eluted with MeOH affording A2a. A2a was further separated by semi-preparative HPLC and eluted with MeOH−H2O (20:80 v/v to 80:20 v/v in 30 min, 2 mL/min), resulting in the isolation of compounds 8, 11, 13, 15 and 20.

Fraction B was chromatographed over a column (3 × 70 cm) of Sephadex LH-20 eluted with MeOH, affording two fractions (B1 and B2). B1 and B2 were further separated by semi-preparative HPLC and eluted with MeOH−H2O (20:80 v/v to 80:20 v/v in 30 min, 2 mL/min) to yield Compounds 6, 7 and 10 from fraction B1 and Compound 16 from fraction B2.

Fraction C was separated over MPLC, by elution with MeOH−H2O (10:90 v/v to 70:30 v/v, 3 mL/min), affording three fractions (C1−C3). C1 was separated by semi-preparative HPLC eluted with MeOH−H2O (20:80 v/v to 80:20 v/v in 30 min, 2 mL/min) to yield compounds 17 and 19. Similarly, C2 and C3 were separated by semi-preparative HPLC eluted with MeOH−H2O (20:80 to 80:20 in 30 min, 2 mL/min) to yield compounds 11 and 14 from fraction C2 and compounds 3 and 4 from fraction C3.
Figure 3: Isolation Scheme for Norway Maple Bark

Norway Maple bark (Acer platanoides) (4.9 kg)

Extracted with MeOH

MeOH Extract (300 g)

Suspended in H2O and partitioned in BuOH and Ethyl Acetate

BuOH Extract (150 g)

Ethyl Acetate Extract (100 g)

MCI Column

Fraction 1 (47.53 g)

Silica Gel Column

Fraction A (6.3 g)

Sephadex LH-20

A1 (209 mg)

Semi-preparative HPLC

Compounds 1, 2, 5, 12, 19

A2 (1.1 g)

A2a (728 mg)

Semi-preparative HPLC

Compounds 8, 11, 13, 15, 20

Fraction B (1.07 g)

Sephadex LH-20

B1 (234 mg)

Semi-preparative HPLC

Compounds 6, 7, 10

B2 (16.7 mg)

Semi-preparative HPLC

Compound 16

Fraction C (12.03 g)

Sephadex LH-20

C18 MPLC

C1 (1.4 g)

Semi-preparative HPLC

Compounds 17, 19

C2 (300 mg)

Semi-preparative HPLC

Compounds 11, 14

C3 (1.2 g)

Semi-preparative HPLC

Compounds 3, 4
Analytical High Performance Liquid Chromatography (HPLC-UV): All analytical HPLC analyses were conducted using a Hitachi HPLC utilizing a Diode Array Detector (DAD) L-2455, L-2130 pump, LH-2300 column oven and LH-2200 auto-sampler. The HPLC operation software used was EZ Chrom Elite, Version: 3.3.2 sp1. For analytical HPLC analyses, all samples (20 µL injection volume; 10 mg/mL concentrations) were filtered (0.22 µM) and analyzed on a Luna C-18 column (Phenomenex; 250 x 4.6 mm i.d., 5µ). The mobile phase, solvent A: 0.1% Trifluoroacetic acid-DI Water, and solvent B: methanol; Gradient % A: initial: 90%, 40 min: 10%, 41 min: 90%; run time 51 min; flow rate 0.75 mL/min; compounds were monitored at wavelengths of 210–600 nm.

α-Glucosidase Inhibitory Assay:

A mixture of 50 µL of different concentrations of individual compounds 1–18, 100, 50, 25, 12.5, 6.25 µg/mL of the test samples and 100 µL of 0.1 M phosphate buffer (pH 6.9) containing yeast α-glucosidase solution (1.0 U/mL) was incubated in 96-well plates at 25 °C for 10 min. After pre-incubation, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min. Before and after incubation, absorbance was recorded at 405 nm by a micro plate reader (SpectraMax M2, Molecular Devices Corp., operated by SoftmaxPro v.4.6 software, Sunnyvale, CA, USA) and compared to that of the control, which had 50 µL buffer solutions instead of the test compounds. The α-glucosidase inhibitory activity was expressed as percent inhibition and was calculated as follows.
Identification of Compounds from Norway Maple Bark: All isolated compounds were identified by examination of their $^1$H and/or $^{13}$C-NMR and mass spectral data and by comparison of these to published literature reports, where available.

Epi-catechin Aldehyde (Aceraldehyde) (19): $^1$H-NMR (CD$_3$OD, Varian 500 MHz):

\[ \delta \text{ 10.1 (1H, s), 7.02 (dd, } J=8.1, 1.8 \text{ Hz), 6.83 (bd, } J= 8.1 \text{ Hz), 6.74 (d, } J=1.8 \text{), 6.74 (d, } J=1.8 \text{ Hz), 5.93 (s), 2.53 (m), 2.41 (dd, } J=5.2 \text{ Hz, 16.6 Hz), 3.99 (dd, } J=6.7 \text{ Hz, 16.0), 4.84 (d, } J=6.3 \text{ Hz),} \]

$^{13}$C-NMR CD$_3$OD $\delta$ (ppm): 191.1, 117.7, 114.6, 144.7, 144.7, 113.6, 129.8, 158.7, 98.9, 104.6, 163.6, 94.1, 165.4, 27.4, 65.0, 79.2. $^1$H-NMR data were consistent with the literature.$^{11}$
Figure 4: Structures of Chemical Constituents Identified in Norway Maple Bark
Figure 5: Structure of Epi-catechin Aldehyde, Aceraldehyde

Figure 6: Key HMBC Correlations of Epi-catechin Aldehyde, Aceraldehyde
Figure 7: Proposed biosynthesis of epi-catechin aldehyde, Aceraldehyde
Results and Discussion:

Compound 19, an off-white powder, was assigned the molecular formula of C_{16}H_{14}O_{7} determined by HRESIMS data to be m/z 318.0740 [M-H]. The presence of 15 carbons on \(^{13}\)C-NMR is characteristic of a flavanone (See figure 5). Three proton signals at \(\delta 7.02, 6.83, \) and 6.74 correlating to the \(^{13}\)C signals at \(\delta 113.7, 117.8, \) and 114.7 ppm to form a dihydroxylated benzene ring. Moreover, the epi-catechin unit was also consistent with literature\(^{20}\) with the exception of a singlet at \(^{1}\)H-NMR \(\delta 10.1 \) ppm. This is a typical chemical shift of an aldehyde, later confirmed by \(^{13}\)C-NMR data showing a peak at \(\delta 191.0 \) ppm. Utilizing heteronuclear multiple-bond correlation (HMBC) (See figure 6), the singlet at \(^{1}\)H 5.93 correlated to C-5 and C-7 and the singlet at \(^{1}\)H-NMR 10.1 correlated to C-7 and C-8, therefore confirming the aldehyde functionality to be at position 8b. The stereochemistry for compound 19 was determined using nuclear overhauser effect spectroscopy (NOESY) showing correlation between H-2 and H-3 at \(\delta 3.99 \) and 4.84 ppm respectively, confirming the stereochemistry of epi-catechin (See figure 6). Although this is the first isolation of this compound from nature, this compound has been synthesized before by Safi et al.\(^{11}\)

The biosynthesis of compound 19 is being proposed as a nucleophilic substitution where methyltransferase attacks C-8 and is methylated by S-adenosylmethionine (SAM) (See figure 7). This is then enzymatically oxidized twice by cytochrome p-450 yielding the aldehyde moiety at carbon 8b. This is initiated by L-methionine being converted to SAM resulting in positively charged sulfur to initiate the nucleophilic substitution reaction. Subsequently, the methyl-building unit is
introduced by a nucleophilic substitution reaction to begin methylation. This reaction requires a nucleophilic carbon; and, thus a phenol with an adjacent carbonyl group is susceptible to methylation.

Apart from compound 19, nineteen known phenolics identified as protocatechuic acid (1), methyl gallate (2), 4-methoxyl 5-hydroxymethyl benzoic 3- O- β- D glucopyranoside (4), gallic Acid 4-methyl ether (5), gallic acid (6), vanillic acid (7), protocatechoic acid (8), benzoic acid, 3,5-dihydroxy-4-methoxy-, methyl ester (9), hydroxyhydroquinone (10), fraxidin (11), fraxetin (12), scopoletin (13), isofraxidin (14), 6,7,8 trihydroxycoumarin (15), catechin (16), epi-catechin (17), cleomiscosin C (18), and benzyl alcohol (20). They were identified on the basis of 1D and 2D NMR and compared to literature when available. Compound 19 was not evaluated due to a limited quantity that was isolated. Compounds 1–18 were evaluated for their α-glucosidase inhibitory activity. Hydroxyhydroquinone (10) and Fraxidin (11) showed moderate activity compared to the control, Acarbose with an IC$_{50}$ of 170 µM. Compounds 10 and 11 had IC$_{50}$ values of 170 µM and 136 µM respectively. However, compounds 1–9, 12–18 inhibitory activities were not active.

In conclusion, this investigation of compounds isolated from Norway maple bark yielded 19 phenolic compounds. After a thorough phytochemical investigation was conducted, compounds were compared to other phytochemicals that previously had been isolated from the hardwood of other species of the Acer genus. These results are consistent with previously reported literature reporting phenolics to be the primary phytochemicals from the Acer genus. Additionally, secondary metabolites are known
not only to be organ specific, they can also be genus specific. Many of the phenolic compounds such as gallic acid,\textsuperscript{8} vanillic acid,\textsuperscript{23} scopoletin,\textsuperscript{19,23} catechin\textsuperscript{20} and other compounds are commonly found in many higher plants. These compounds have been investigated for their biological activity as free radical scavengers.\textsuperscript{8,20,23,24}

Furthermore, another compound isolated from other \textit{Acer} species is cleomiscosin C. It is a coumarolignan that has been isolated from the hardwood of trees including the Sugar maple and the Korean maple (\textit{Acer okamotoanum}).\textsuperscript{6,25} Cleomiscosin C has also been previously isolated from the stem wood of \textit{Aquilaria agallocha}, and seeds of \textit{Cleome viscoas}.\textsuperscript{6} This coumarolignan has also been synthesized using fraxetin as a starter unit,\textsuperscript{21} and shown to have significant antioxidant potential.\textsuperscript{25,26}

This provides evidence that compounds may be organ specific. Moreover, this also suggests these non-edible plant parts should be investigated for their phytochemicals, specifically those that, due to potential health benefits, could be used in nutraceutical formulations.
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First Isolation of Aceraldehyde from Norway Maple (*Acer platanoides*) Bark

Raed Omar, Tao Yuan, Liya Li, Hang Ma, and Navindra P. Seeram*
A-type Proanthocyanidins Cinnamtannins B1 and D1 Isolated from Highbush Blueberry (Vaccinium corymbosum) Roots

Raed Omar, Amanda L. Cirello, Tao Yuan, Liya Li, and Navindra P. Seeram *

Bioactive Botanical Research Laboratory, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island 02881, United States

*Corresponding Author. (N.P.S.) Email: nseeram@uri.edu. Phone: (401) 874-9367; Fax: (401) 874-5787;

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Abstract: Highbush blueberry fruits and flowers were shown to inhibit $\alpha$-amylase and $\alpha$-glucosidase enzymes, relevant to type-2 diabetes management. Blueberry fruits and flowers have also been investigated for their phytochemicals. However, the roots have not yet been investigated. Here we report the first isolation and elucidation of cinnamotannins B1 and D1 isolated from the highbush blueberry roots along with three other known phenolic compounds.

KEYWORDS: Vaccinium corymbosum, highbush blueberry, roots, phenolics
**Introduction:** Roots play a very important role for a plant. They are responsible for the plant’s absorption of minerals, oxygen and water. In winter months, the roots reserve food needed by the tree to produce spring foliage. They are also responsible for anchoring the plant/tree above ground. Therefore, it is important to keep the portion above ground healthy by ensuring adequate water and rich soil for the roots to continue their functions. However, because the roots are exposed to many different environmental conditions to which the rest of the plant is not, they most likely produce different phytochemicals compared to the other organs of the plant. By maintaining healthy roots, the plant is able to produce better quality products such as berries.

Berry fruits are widely consumed in our diet and have attracted much attention due to health benefits for which they have been investigated extensively. Berries contain a diverse range of phytochemicals with biological properties which act as antioxidants, and anticancer and anti-inflammatory agents. Flavonols have been of particular interest as they are known antioxidants agents that may inhibit the onset of coronary heart disease.

The phenolic content in berries is affected by the degree of maturity at harvest, cultivar, environmental conditions, storage conditions and processing. Polyphenols, abundant in blueberries, have been seen to produce favorable nootropic, antioxidant and anti-inflammatory effects. One of the most popularly consumed berries is the blueberry. Blueberries are consumed for reasons beyond their taste. Research has suggested blueberries have many preventative properties. The fruits, leaves and flowers are rich sources of vitamins A and C, and nutrients such as flavonoids and anthocyanins. Furthermore, highbush blueberry flowers and fruits were shown to
inhibit α-amylase and α-glucosidase enzyme activity, relevant to type-2 diabetes management.\textsuperscript{3,7,10}

Phytochemical isolation efforts on various plant parts reveal their potential to contain novel compounds. Many studies have been conducted on the berries and flowers of the highbush blueberry. However, the roots have not been investigated.\textsuperscript{3,7}

The objectives of this project were to isolate and elucidate secondary metabolites in highbush blueberry roots. This is the first phytochemical and biological study of roots of the highbush blueberry species.

**General Experimental Procedures:**

\textsuperscript{1}H and \textsuperscript{13}C Nuclear Magnetic Resonance (NMR) data were recorded on a Varian 500 MHz instrument with TMS as internal standard. Electrospray Ionization Mass Spectral (ESI-MS) data were acquired on a Q-Star Elite (Applied Biosystems MDS) mass spectrometer equipped with a Turbo Ionspray source and were obtained by direct infusion of pure compounds. The UV spectra were measured on a SHIMADZU UV-2550 UV-visible spectrophotometer. Medium-pressure liquid chromatography (MPLC) separations were carried out on pre-packed C18 columns connected to a DLC-10/11 isocratic liquid chromatography pump (D-Star Instruments, Manassas, VA) with a fixed-wavelength detector. High-performance liquid chromatography (HPLC) was performed on a Hitachi Elite LaChrom system consisting of a L2130 pump, L-2200 auto-sampler and an L-2455 Diode Array Detector, all operated by EZChrom Elite software. All solvents were of either ACS- or HPLC-grade and were purchased from Wilkem Scientific (Pawtucket, RI).
Isolation Scheme for Highbush Blueberry Roots

**Figure 8: Isolation Scheme for Highbush Blueberry Roots**

**Extraction:**

The roots of the highbush blueberry species (*Vaccinium corymbosum* var. “Jersey”) were collected locally from Morgan farms (North Kingstown, RI, USA) in May 2009. Voucher specimens 16JPM51-VCJ51309FL are deposited in the Heber-Youngken Garden and Greenhouse at the College of Pharmacy, University of Rhode Island (Kingston, RI, USA). The roots (400 g, fresh weight) were extracted exhaustively with MeOH (3 × 4.3 L) at room temperature to yield a dried MeOH extract (80 g). A portion of the extract (77 g) was re-suspended in H₂O (750 mL) and partitioned with EtOAc (3 × 750 mL) to yield a dried EtOAc extract (13.5 g).
Isolation of Compounds:

The EtOAc extract (13 g) was chromatographed on an MCI gel (MeOH–H2O, 50:50 to 90:10) to yield fraction 1 (See figure 8). Fraction 1 was then chromatographed on an LH-20 column yielding three sub-fractions (A–C), which were combined based on analytical HPLC analyses. Fraction A was separated by semi-preparative HPLC eluted with MeOH/H2O (30/70, v/v; 2.0 mL/min) to yield gallic acid (1). Fraction B was then also separated by Sephadex LH-20 (3.5 × 120 cm) eluted with MeOH to yield sub-fractions B1 and B2. Fraction B1 was further separated by semi-preparative HPLC eluted with MeOH/H2O (45/55, v/v; 2.0 mL/min) to yield catechin (2) and epi-catechin (3). Fraction C was chromatographed and eluted through a Sephadex LH-20 (3.5 × 120 cm) with MeOH to afford Fraction C1. Fraction C1 was further separated by semi-preparative HPLC eluted with MeOH/H2O (45/55, v/v; 2.0 mL/min) to yield cinnamtannin B1 (4) and cinnamtannin D1 (5) (See Figure 8).

α-Glucosidase Inhibitory Assay:

A mixture of 50 µL of different concentrations of compounds 1-5: (100, 50, 25, 12.5, 6.25 µg/mL) of the test samples and 100 µL of 0.1 M phosphate buffer (pH 6.9) containing yeast α-glucosidase solution (1.0 U/mL) was incubated in 96-well plates at 25 °C for 10 min. After pre-incubation, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min. Before and after incubation, absorbance was recorded at 405 nm by a microplate reader (SpectraMax M2, Molecular Devices Corp., operated by SoftmaxPro v.4.6 software,
Sunnyvale, CA, USA) and compared to that of the control, which had 50 µL buffer solutions instead of the test compounds. The α-glucosidase inhibitory activity was expressed as percent inhibition and was calculated as follows:

\[
\% \text{ inhibition} = \left( \frac{\Delta \text{Abs}_{\text{control}} - \Delta \text{Abs}_{\text{sample}}}{\Delta \text{Abs}_{\text{control}}} \right) \times 100
\]

**Identification of Compounds:**

The isolated compounds (chemical structures shown in Figure 1) were identified by a combination of 1D and 2D-NMR and mass spectral data and by comparison of these to published literature reports where available. The $^1$H-NMR data for all the compounds are listed below as well as the $^{13}$C NMR.
Figure 9: Compounds Isolated from the Highbush Blueberry Roots
Figure 10: Cinnamtannins B1 and D1
| Ring | No | δC m   | δH m (J/Hz)         |
|------|----|--------|---------------------|
| C    | 2  | 100.09, C |                     |
|      | 3  | 67.23, CH | 3.88, m            |
|      | 3  |         | 5.28, s            |
|      | 4  | 28.8, CH | 4.29, d (3.5)      |
| A    | 5  | 156.7, C |                     |
|      | 6  | 96.6, CH | 5.90, d (2.2)      |
|      | 7  | 157.3, C |                     |
|      | 8  | 96.8, CH | 5.89, d (2.2)      |
|      | 9  | 154.1, C |                     |
|      | 10 | 104.9, C |                     |
| B    | 1' | 131.8, C |                     |
|      | 2' | 115.5, CH | 7.03, d (2.1)      |
|      | 3' | 145.4, C |                     |
|      | 4' | 146.2, C |                     |
|      | 5' | 115.7, CH | 6.77, d (8.3)      |
|      | 6' | 119.4, CH | 6.88, dd (8.3, 2.1) |
| F    | 2  | 78.8, CH | 5.13, brs          |
|      | 3  | 72.0, CH | 3.78, m            |
|      | 4  | 38.2, CH | 4.48, brs          |
| D    | 5  | 155.7, C |                     |
|      | 6  | 95.0, CH | 5.95, s            |
|      | 7  | 151.08, C |                   |
|      | 8  | 106.5, C |                     |
|      | 9  | 151.8, C |                     |
|      | 10 | 106.7, C |                     |
| E    | 1' | 131.8, C |                     |
|      | 2' | 115.5, CH | 6.94, d (1.8)      |
|      | 3' | 145.3, C |                     |
|      | 4' | 145.5, C |                     |
|      | 5' | 116.1, CH | 6.72, d (8.4)      |
|      | 6' | 119.4, CH | 6.93, dd (8.4, 1.8) |
| I    | 2  | 78.5, CH | 4.95, brs          |
|      | 3  | 64.7, CH | 4.19, m            |
|      | 4  | 28.8, CH<sub>2</sub> | 2.78, dd (16.4, 4.4) |
|      | 4  |         | 2.61, dd (16.4, 4.6) |
| G    | 5  | 155.5, C |                     |
|      | 6  | 96.7, CH | 5.82, s            |
|      | 7  | 154.12, C | 8.1, s OH         |
|      | 8  | 107.6, C |                     |
|      | 9  | 155.1, C |                     |
|      | 10 | 99.8, C  |                     |
| H    | 1' | 131.6, C |                     |
|      | 2' | 116.1, CH | 6.94, d (1.8)      |
|      | 3' | 145.4, C |                     |
|      | 4' | 145.4, C |                     |
|      | 5' | 116.1, CH | 6.72, d (8.4)      |
|      | 6' | 119.9, CH | 6.87, dd (8.4, 1.8) |
### Table K: δ \(^1\)H and \(^{13}\)C Data for Compound 5

| Ring | no | δc m  | δH m (J/Hz)         |
|------|----|-------|---------------------|
| C    | 2  | 100.09, C |                     |
|      | 3  | 67.25, CH | 3.84, m             |
|      | 3  |         | 5.32, s             |
|      | 4  | 28.8, CH | 4.28, d (3.1)       |
| A    | 5  | 156.6, C |                     |
|      | 6  | 96.3, CH | 5.90, d (2.2)       |
|      | 7  | 157.7, C |                     |
|      | 8  | 96.8, CH | 5.89, d (2.2)       |
|      | 9  | 154.2, C |                     |
|      | 10 | 105.2, C |                     |
| B    | 1' | 132.4, C |                     |
|      | 2' | 115.8, CH | 7.03, d (1.8)       |
|      | 3' | 145.5, C |                     |
|      | 4' | 146.3, C |                     |
|      | 5' | 115.7, CH | 6.77, d (8.3)       |
|      | 6' | 120.0, CH | 6.88, dd (8.2, 2.1) |
| F    | 2  | 78.7, CH | 5.21, brs           |
|      | 3  | 72.1, CH | 3.80, m             |
|      | 4  | 38.3, CH | 4.46, brs           |
| D    | 5  | 156.6, C |                     |
|      | 6  | 95.8, CH | 5.95, s             |
|      | 7  | 151.05, C |                |
|      | 8  | 106.6, C |                     |
|      | 9  | 151.7, C |                     |
|      | 10 | 106.7, C |                     |
| E    | 1' | 132.4, C |                     |
|      | 2' | 115.8, CH | 6.97, d (2.1)       |
|      | 3' | 145.5, C |                     |
|      | 4' | 145.8, C |                     |
|      | 5' | 116.2, CH | 6.73, d (8.3)       |
|      | 6' | 120.0, CH | 6.94, dd (8.31, 2.1) |
| I    | 2  | 80.1 CH  | 4.48, d (5.1)       |
|      | 3  | 66.1 CH  | 3.94, m             |
|      | 4  | 25.8 CH\textsubscript{2} | 2.44, dd (16.2, 4.6) |
|      | 4  |         | 2.41, dd (16.2, 5.4) |
| G    | 5  | 155.8, C |                     |
|      | 6  | 96.7, CH | 5.80, s             |
|      | 7  | 154.2, C |                     |
|      | 8  | 108.6, C |                     |
|      | 9  | 155.1, C |                     |
|      | 10 | 100.0, C |                     |
| H    | 1' | 131.6, C |                     |
|      | 2' | 116.2, CH | 6.76, d (1.8)       |
|      | 3' | 145.5, C |                     |
|      | 4' | 145.4, C |                     |
|      | 5' | 116.2, CH | 6.68, d (8.1)       |
|      | 6' | 120.0, CH | 6.81, dd (8.1, 1.8) |
**Results and Discussion:** Five phenolics, Gallic Acid (1), Catechin (2), Epi-catechin (3), Cinnamtannin B-1 (4), and Cinnamtannin D-1 (5) were isolated from highbush blueberry roots. Although gallic acid, catechin and epi-catechin are ubiquitous compounds found in many higher plants, cinnamtannins are not as common. They have been isolated from the roots of several plants including the *Cinnamomum cassia*, *Pottsia laxiflora* and the leaves of *Cinnamomum subavenium* and *Machilus philipinensis*.

Compound 4 showed a molecular formula of C_{45}H_{36}O_{18} determined by HRESIMS data to be m/z 864.1902 [M-H]. It assumed to be a trimeric proanthocyanidin (PAC) due to the presence of 45 carbons composed. The $^1$H-NMR spectra exhibited the presence of an A type unit from the signals at δ 3.29 and 4.15 (each d, $J$ = 3.5 Hz) and were assigned to H-3 and H-4 of the C ring. This double link was supported from the carbon signal at δ 99.9 for C-2 on the C ring of the $^{13}$C spectra. Key HMBC correlations included H-4 of the C ring to C-7, C-8 and C-9 of the D ring, H-3 of ring F to C-7 and C-8 of ring G (See Figure 11). The NMR data of cinnamtannin D1 were similar to those of cinnamtannin B1 with the exception of the resonances in the GHI moiety, which appeared at $^1$H-NMR δ 3.96 (d, $J$=9.1 Hz), 3.69 (m, $J$=10.1, 9.1, 6.0 Hz), 3.04 (m, $J$=16.1, 10.1 Hz), 2.41, (dd, $J$=16.1, 10.1 Hz) and $^{13}$C-NMR δ 80.1, 66.1 and 25.8 indicating the presence of a catechin moiety (See Tables J and K).

Our identification of phenolic compounds in the highbush blueberry species is consistent with previous reports of phenolics being the major constituents in plant parts of the lowbush blueberry species.
The biological activities of these phenolics have been previously investigated, including their antioxidant activity, antibacterial, anticarcinogenic and α-glucosidase inhibition.\textsuperscript{2-10}

In this study, our laboratory investigated the α-glucosidase inhibitory activity of cinnamottannins B1 and D1. However, their activity was not detected. The antioxidant, antibacterial and α-glucosidase inhibition of cinnamottannins B1 and D1 were also previously investigated.\textsuperscript{12,13} In keeping with reports from previous literature, Cinnamottannins B1 and D1 were found inactive in the α-glucosidase inhibitory assay.
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Figure 11: Key HMBC correlations (H \(\rightarrow\) C) of Cinnamtannin B1.
TOC Graphics

A-type Proanthocyanidins Cinnamotannins B1 and D1 Isolated from Highbush Blueberry (Vaccinium corymbosum) Roots

Raed Omar, Amanda L. Cirello, Tao Yuan, Liya Li, and Navindra P. Seeram *
Topical Maple Syrup Formulation Acts as a Humectant

Raed Omar and Navindra P. Seeram*

Bioactive Botanical Research Laboratory, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island 02881, United States

*Corresponding Author. (N.P.S.) Email: nseeram@uri.edu. Phone: (401) 874-9367; Fax: (401) 874-5787;

This manuscript is written to be submitted to World Academy of Science, Engineering and Technology
Abstract: This is the first investigation utilizing maple syrup as an active ingredient in a topical cream. This study was designed to formulate a topical skin-care cream of maple syrup. A topical cream formulation containing 12% maple syrup was developed by creating an emulsion. The evaluation parameters consisted of color, smell, type of emulsion, phase separation, liquefaction, and pH. The stability of the formulations were evaluated on cream samples kept at 8°C, 25°C, 40°C, 40°C with 45% relative humidity and 40°C with 75% relative humidity. No significant differences were observed between day zero and day 21 for the pH of both the base and maple syrup formulations (p>0.05). The newly formulated maple syrup cream is suitable for improved skin hydration levels and reducing trans-epidermal water loss in people with dry skin.
Introduction:

An emulsion is a preparation of two immiscible substances. Creams are an example of a common emulsion with a water phase and an oil phase. For these phases to be made miscible, an emulsifying agent is employed (i.e. bees wax). A water in-oil emulsion is commonly used for the treatment of dry skin. Active ingredients with specific biological properties such as antibacterial, antiviral, and anti-inflammatory properties, may enhance a formulation for a desired cosmetic effect. Cosmetic formulations are commonly enhanced with antioxidants.

Honey has been investigated for its wound-healing properties; and, its powerful healing abilities have been attributed to its sugar content. Flavonoid-rich plants have been researched for their wound-healing ability and anti-aging potential. An emulsion with high sugar content in conjunction with a high polyphenolic profile may assist with transepidermal water loss contributing to dry skin. Honey and maple syrup have similar phytochemical constituents composed primarily of sugar.

The phytochemicals of maple syrup have been investigated along with vitamin and sugar analysis. Maple syrup, like honey, is composed primarily of sugar. Sugar content has been shown to assist with increasing skin elasticity to help with proper wound closure. Maple syrup’s combination of phytochemicals and high sugar content suggests it can act as a potent anti-aging moisturizing formulation.

Moreover, Cooper et al reported that a minimum of 29% sugar content is required for inhibition of Staphylococcus aureus. Furthermore, they reported better inhibition at higher concentrations, compared to the lower concentrations of honey. Sugar may play a role in inhibiting bacterial growth.
Hyaluronic acid (HA) consists of disaccharide chains made from modifications of the monosaccharide glucose.\textsuperscript{11} It is believed that glucose derived from sugar may be converted into hyaluronic acid at the wound surface, forming an extracellular matrix.\textsuperscript{9} HA is thought to aid in dermis healing. It is believed to assist in activating the inflammatory response, promoting cell proliferation, migration, and promoting re-epithelization.\textsuperscript{12} Therefore, HA may assist in moisturizing in topical formulation.

Due to the increased awareness of the adverse effects of synthetic compounds, an investigation of the moisture retentive properties of a maple syrup based emulsion was conducted compared to a base without maple syrup. Maple syrup is natural and free of any coloring or additives. It is boiled down directly from tree sap, which is harvested from the maple tree towards the end of winter. Maple syrup is not processed and contains vitamins and minerals, including calcium, potassium, sodium and copper suggesting it may be a candidate for an active ingredient as part of a moisturizing formula.\textsuperscript{5,13}

**General Experimental Procedures:** 100\% Pure Maple Syrup was purchased from A&P in Saddle Brook, New Jersey. A digital pH-Meter by General Tools & Instruments Company LLC.

**Preparation of Water-to-Oil Emulsion**

In this study, a water-to-oil emulsion was prepared by the addition of aqueous phase to the oily phase with continuous agitation. Oily phase consisted of paraffin oil (16\%) and surfactant ABIL-EM 90 (4\%) heated up to 75°C. Water soluble ingredients were heated to 75°C and the maple syrup extract (12\%) was then added to the experimental
cream. The aqueous solution was added to the oily phase while stirring was continued at 3,500 rpm by the high-shear mixer for about 15 minutes until complete aqueous phase was added. After the complete addition of the aqueous phase, the speed of the mixer was reduced to 1,500 rpm for homogenization, for a period of 5 minutes, and the emulsion was then left to cool at room temperature. The base was prepared using the same method without the addition of the maple syrup.

**Physical analysis of formulations:**

The emulsion was analyzed based on color, thickness, look, feel and emulsion properties.

**pH determination**

pH value of freshly prepared emulsion and emulsions kept at different conditions were determined by a digital pH-Meter by General Tools & Instruments Company LLC.

**Stability tests**

Stability tests for the emulsions were performed in several different conditions. These storage conditions were analyzed to see whether they had an effect on the emulsion. Tests were performed on samples kept at 8°C (in a refrigerator), 25°C (in incubator), 40°C (in incubator), 40°C (in incubator) with 45% relative humidity and 40°C (in incubator) with 75% relative humidity. Physical characteristics were also monitored daily. Photo-oxidation was also tested via exposure to UV rays 100–400 nm in a dark box for one month and monitored for physicochemical changes.
Results & Discussion:

It is important that formulations are stabilized to help determine shelf life. Products should have a shelf life of at least one year at room temperature and under varying external factors. The objective is to determine if the emulsion is stable before it separates into two layers visible by the naked eye. The base and maple syrup formulations were evaluated at 8°C, 25°C, 40°C and 40°C and 75%RH. Stability was achieved after three weeks stored in variable conditions. The physical appearance and smell of the experimental maple syrup formulation and base formulation were not altered. There was no crystallization observed within the container or after application to the skin. Information about the emulsion and the temperature’s effect on the formulation, the emulsion underwent temperature cycles from 5 to 40 °C within 24 h for four weeks. This is frequently used in industry as an accelerated stability test to predict long-term stability.

Figure 12: pH of Formulations

It is important that formulations are stabilized to help determine shelf life. Products should have a shelf life of at least one year at room temperature and under varying external factors. The objective is to determine if the emulsion is stable before it separates into two layers visible by the naked eye. The base and maple syrup formulations were evaluated at 8°C, 25°C, 40°C and 40°C and 75%RH. Stability was achieved after three weeks stored in variable conditions. The physical appearance and smell of the experimental maple syrup formulation and base formulation were not altered. There was no crystallization observed within the container or after application to the skin. Information about the emulsion and the temperature’s effect on the formulation, the emulsion underwent temperature cycles from 5 to 40 °C within 24 h for four weeks. This is frequently used in industry as an accelerated stability test to predict long-term stability.
The pH of the base and maple syrup were 5.9 and 5.2 respectively. The newly formulated maple syrup cream is suitable for improved skin-hydration levels. By reducing trans-epidermal water loss in people with dry skin, and decreasing the appearance of wrinkles, maple syrup cream acts a humectant. Previous literature supports the hypothesis of topical use of a high-sugar polyphenolic-rich active ingredient to increase moisture retention and wound healing, or as a potent antioxidant.  

Antioxidants protect human skin from free radicals produced by UV radiation; and, flavonoids are known to be potent antioxidants and photo-protective agents. Additionally, trans-epidermal water loss results in loss of collagen regeneration, and the loss of collagen causes wrinkles.

The benefits of topically applied sugar have been investigated and show improvements on tensile strengthening. Moreover, sugar’s antibacterial capabilities make it ideal when used with potent antioxidants for anti-aging capabilities.

Moreover, vitamins including as vitamin A (retinol), vitamin E (α-tocopherol), and Vitamin C (ascorbic acid) are known to assist in wound healing. Topical application of vitamin C has been shown to elevate cutaneous levels of vitamin C, which correlates with the antioxidant protection of the skin protecting the skin from UVB damage. Additionally, vitamin C was observed to play an important role increasing type I and type III collagen levels. Vitamin C has been shown to be a potent anti-oxidant removing dead skin cells with vitamins A and E, the formulation assists by removing age spots and healing wounds, ultimately rejuvenating the skin.
The polysaccharide and flavonoid-rich composition of maple syrup may impart moisturizing properties to the skin.
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TOC Graphics

Topical Maple Syrup Formulation Acts as a Humectant

Raed Omar and Navindra P. Seeram*
Epi-catechin Aldehyde (Aceraldehyde): $^{13}$C-NMR
Cleomiscosin C: $^1$H-NMR
Cleomiscosin C: $^{13}\text{C}$-NMR
Cinnamtannin B1: $^1$H-NMR
Cinnamamin B1: 13C-NMR
Cinnamamin D1: H-NMR
