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MAE of phenolic compounds from blueberry leaves and comparison with other extraction methods

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ABSTRACT

Blueberry leaves are a prospective source of phenolic compounds which can be obtained by microwave assisted extraction leading to higher yield in shorter period of time and with lower solvent consumption when compared to solvent extraction. During this study, three experiments with microwave assisted extraction, were separately designed and conducted using highbush blueberry (variety Bluetta) leaves collected during the late fall season. The yields of different compounds obtained during these experiments with microwave extraction were compared with ultrasonic extraction for one hour and 24-h room temperature extraction. The first microwave assisted extraction experiment was conducted to streamline the combination of ethanol and citric acid concentration for further analysis of microwave as a potential extraction method for phenolic compounds and anthocyanins from blueberry leaves. During the second experiment the effect of microwave power level (10–20% absolute power level) and time of extraction (4–16 min) on total phenolics, total anthocyanins and chlorogenic acid was studied which was further used to design the third experiment consisting of a face-centred central composite design with varying factors namely power level and time, each at 3 levels. All the experimental combinations for the 2nd and 3rd experiments were conducted with 80 ml of solvent consisting of 30% ethanol and 1.5 M citric acid combination in ratio of 97:3 (v/v). The total anthocyanins and chlorogenic acid extracted during the study were between 2.321–2.636 mg malvidin 3-glucoside/g dry matter and 49.34–52.66 mg chlorogenic acid/g dry matter respectively, not showing a huge variation between highest and lowest yields, however both compounds with a noteworthy yield. For total phenolics extraction, both microwave power and time of microwave application were observed to be statistically significant factors and the yield of total phenolics was found to be much higher (in the range of 92.719–128.76 mg GAE/g dry weight) than for 24-h extraction at room temperature (average 89.164 mg GAE/g dry weight) and 1 h extraction by sonication (average 97.77 mg GAE/g dry weight) with the same solvent combination.

1. Introduction

Blueberry is a popular fruit of North America and an important high value crop. Blueberries are important source of many beneficial phenolic compounds including anthocyanins (the pigments responsible for the colour of the fruit) and chlorogenic acid. Blueberry fruits are increasingly known for their health beneficial effects including anti-diabetic, anti-cancerous, anti-bacterial, antioxidant effects and positive effects against vascular and neurodegenerative disorders (Chatterjee et al., 2004; Grace et al., 2009; Li et al., 2012; Martineau et al., 2006; Neto, 2007; Park et al., 2011; Ramassamy, 2006; Seeram et al., 2006). Other than the fruits, recently blueberry leaves have also become the focus of many research studies (Cyboran et al., 2013; Duy, 1999; Ehlenfeldt and Prior, 2001; Hicks et al., 2012; Hokkanen et al., 2009; Kim et al., 2010; Martz et al., 2010; Naczk et al., 2003; Vyas et al., 2013). The research has been principally done on lowbush blueberries, bilberries and rabbiteye blueberries; while research on different varieties of commercial highbush blueberries is less available. Blueberry leaves (highbush and lowbush) have been reported to be a good source of chlorogenic acid which is known for its antioxidant properties (Hicks et al., 2012; Kim et al., 2010). Blueberry leaves, like the leaves of many other North American plants' change colour during the Fall season and can be a good source of anthocyanins, however research in this area is rare and there are very few reports on this subject (Duy, 1999). Phenolic compounds are vital secondary

Abbreviations: MAE, microwave assisted extraction; GAE, gallic acid equivalent; HPLC, high performance liquid chromatography; MAP, total monomeric anthocyanin content; CCD, central composite design; M 3-G, malvidin 3-glucoside; equiv., equivalent; ANOVA, analysis of variance; w, weight; v, volume.

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compounds and are present in all plants; however they vary in content depending on different species, cultivation practices, soil and other components affecting plant growth. There are many available reports on the quantification of phenolic compounds from blueberry fruits, while there are only rare reports on phenolic content of leaves. These leaves can be a cheap and abundant source of the constituent compounds that can be used as standards in analytical research, and a chief source of antioxidants which can be further used in a variety of foods or other applications. These leaves were traditionally used for the preparation of tea by native Americans, which can be categorized as an extract of sort and used and promoted as a remedy for various health concerns (Eck, 1988; Gough, 1994).

For extraction of useful phytochemicals, modern extraction methods like microwave assisted extraction can be very effective. Microwave assisted extraction requires less solvent, less time and is considered environmentally friendly with little to no CO_2 emission (Routray and Orsat, 2012). Microwave assisted extraction works via two mechanisms of energy transfer, namely dipole rotation (reversal of dipoles) and ionic conduction (movement of charged ions present in the solute and solvent) (Kubrakova and Toropchenova, 2008). Polar solvents are considered more effective in the case of microwave extraction in terms of effectively absorbing the electrical energy. However, good performance of a solvent under microwave environment depends on both the dielectric constant and dissipation factor, which are expected to be high for the solvents used to achieve effective impact of microwave. Absorption of microwave energy by biological materials leads to the build-up of pressure within the cellular material leading to eventual splitting of the cellular structure with release of its content which increases the efficiency of the process, which is further supported by the careful selection of an adequate solvent to interact with the components to be extracted (Kratchanova et al., 2004; Routray and Orsat, 2012). Microwave assisted extraction has been applied for the extraction of various phenolic compounds from different types of biological samples such as sour cherry, flax seed, potato peel, *Citrus lemon* peels, cherry laurel fruits and leaves and blueberry powder (Dahmoune et al., 2013; Elez Garofulić et al., 2013; Karabegović et al., 2014; Nemes and Orsat, 2010; Proestos and Komaitis, 2008; Singh et al., 2011; Zheng et al., 2013). Some of the common solvents of extraction of phenolic compounds include methanol and ethanol (Nour et al., 2014), though for extraction of anthocyanins, methanol with HCl is one of the most common solvent mixture (Giusti et al., 2005). However, for nutraceutical purposes food application, or extraction, ethanol is preferred along with milder organic acids, preferred to prevent the degradation of important phenolic compounds such as anthocyanins and to remain food grade. During a previous study, it was reported that ethanol and citric acid combinations have noteworthy dielectric properties with a dissipation factor higher than 0.1 which supports its possible effectiveness as a solvent under microwave conditions (Routray and Orsat, 2014). Ethanol has been used for phenolic microwave extraction with a variety of biomaterials such as grape seed and coffee (Krishnaswamy et al., 2013; Pavlović et al., 2013).

Extraction of phenolic compounds at low temperature or room temperature has been applied before as a traditional method of extraction in several cases (Duy, 1999; Gao and Mazza, 1996; Giusti et al., 2005; Nicoué et al., 2007). Sonication is one of the recent extraction method, where sound waves are applied to a solvent plant material mixture for efficient extraction of phenolic compounds (Cheok et al., 2013; Li et al., 2005; Ma et al., 2009; Wang et al., 2008).

In the current study, phenolic compounds were extracted from highbush blueberry (*Vaccinium corymbosum* variety Bluetta) leaves using microwave assisted extraction at a fixed power for a determined time period with solvent combinations of ethanol, citric acid and water. Microwave assisted extraction was compared to 24-h room temperature extraction and 1 h ultrasonic extraction in terms of quantity of respective compounds extracted. Total phenolics content, total monomeric anthocyanins content and chlorogenic acid were quantified during this study.

Table 1
Factors and their levels used for different experiments during the study.

Factors	Levels		
Experiment 1: 2 × 2 full factorial design			
Ethanol concentration (%, v/v)	15	30	
Acid volume: total solvent volume	0.02	0.03	
Experiment 2: 2 × 2 full factorial design with centre point (2 × 2 factorial points + 1 centre point)			
Microwave power (%)	10	15	20
Time (min)	4	10	16
Experiment 3: face centred CCD (2 × 2 axial points + 2 × 2 factorial points + 6 experiments with centre point)			
Microwave power (%)	10	15	20
Time (min)	4	14	24

Note: All the experimental combinations were replicated twice other than the 6 experiments with combination of centre point for Experiment 3.

2. Materials and methods

2.1. Chemicals

Chemicals used during the study were of analytical grade. HPLC grade water was prepared using Simplicity™ Water Purification System (Millipore, USA). Ethanol was obtained from Commercial alcohols (The Industrial and Beverage Alcohol Division of Greenfield Ethanol Inc., Brampton, Ontario, Canada). Citric acid, sodium carbonate, methanol, sodium acetate, HCl and potassium chloride were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Folin–Ciocalteu reagent, gallic acid and orthophosphoric acid were obtained from Sigma–Aldrich (St. Louis, MO, USA). Chlorogenic acid standard was obtained from Acros Organic (NJ, USA). Respective concentrations of chemicals were prepared using the corresponding standards and HPLC grade water.

2.2. Preparation of sample

Northern high-bush blueberry (*V. corymbosum*) variety "Bluetta" leaves were collected in October 2011 from a private farm, Bleuetière Sylvie Rémillard situated in Franklin, Québec, Canada. Though the soil variety in Quebec varies a lot, soil found in this area can be mainly categorized as brown podzolic. Overall the weather in this region varies from warm to humid in summer and very cold in winter with lots of snow. The leaves were immediately frozen at −18 °C until drying. The leaves were dried using a freeze drier (Freezone® 2.5 l Freeze Dry System, Labonco Corporation, Kansas City, MO, USA) for 48 h and equilibrated in a dessicator. The average moisture content of the harvested leaves was found to be 50.86% (wet basis). Following drying the leaves were ground to a fine powder, which was passed through sieve number 18 (size 1 mm) to obtain a homogeneous sample size.

2.3. Microwave extraction, ultrasonic extraction and 24-h extraction

For MAE, approximately 0.5 g sample of blueberry leaves' powder was taken in a tubular semi-spherical bottomed flask. Eighty ml of solvent was added to the flask. The solvent mixture consisted of solutions of ethanol (15 or 30%) and citric acid (1.5 M), in a proportion varying according to the experimental design (Table 1). The sample solvent mixture was heated by application of microwave