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An improved method for extraction of nutraceutically important polyphenolics from *Berberis jaeschkeana* C.K. Schneid. fruits

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ABSTRACT

Berberis jaeschkeana fruits, source of nutraceutically important polyphenolics were investigated. A total of 32 experimental run were conducted under Plackett-Burman and central composite design. Microwave power, methanol and HCl concentration significantly ($p < 0.05$) affect extraction of polyphenols under linear, quadratic and interactive effect. The model showed good fitness with significant ($p < 0.05$) model *F-value* and a non-significant lack of fit. Under optimum microwave assisted extraction (MAE) condition the total phenolics, flavonoids, tannins and antioxidant activity were in closed context with predicted values. As compared to ultrasonic (UAE) and maceration extraction (ME), MAE showed significantly ($p < 0.05$) higher recovery of TP, TF and FRAP antioxidant activity. HPLC-DAD analysis detects a total of 10 polyphenolic compounds under MAE as compared to 9 under UAE and ME. Designing of MAE conditions showed promising results for polyphenolic antioxidants extraction as revealed by higher yield with lesser time and solvent consumption, which can contribute in green extraction technology and its application in nutraceutical industry.

1. Introduction

Wild fruits and berries have been consumed as a source of vitamins, minerals and nutrient supplements (Singh, 2011; Bhatt, Rawat, Badhani, & Rawal, 2017) and are used in the preparations of energy drinks, antioxidant and multivitamin pills, natural flavor, wine, color and food ingredients (Ferruzzi, 2003; Pandya, Solanki, Maniar, Gurav, & Bhatt, 2011). The market of natural antioxidants is increasing exponentially every year and demand in Asia Pacific region is much higher as compared to other regions (Antioxidant market, 2015). This excessive use of natural antioxidants might be due to the fact that these are clinically proven, effective against

Abbreviations: TP, Total phenolics; TT, Total tannins; TF, Total flavonoids; ABTS, 2,2-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid); FRAP, Ferric reducing antioxidant power; DPPH, 2,2-Diphenyl-1-picryhydrazyl; TPTZ, 2,4,6-Tripyridyl-s-triazine; RSM, Response surface methodology; PBD, Plackett-Burman Design; CCD, Central composite design; GAE, Gallic acid equivalent; TAE, Tannic acid equivalent; QE, Quercetin equivalent; AAE, Ascorbic acid equivalent; HPLC, High performance liquid chromatography; DAD, Diode array detection; MAE, Microwave assisted extraction; UAE, Ultrasonic assisted extraction; ME, Maceration extraction; ANOVA, Analysis of variance; DNA, Deoxyribonucleic acid; ROS, Reactive oxygen species.

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cancer, cardiovascular, neurodegenerative and aging related oxidative problems (Nabavi et al., 2013; Subash et al., 2015; Shahidi & Ambigaipalan, 2015; Barreca et al., 2011) and their possible mechanism of action have been explored in number of studies (Suganthy, Devi, Nabavi, Braidy, & Nabavi, 2016; Miltonprabu et al., 2016; Vacca et al., 2016).

Wild fruits and berries from Himalaya have been widely consumed and explored for the presence of vitamins, minerals, natural antioxidants, secondary metabolites, etc. and thus considered as nutraceutically important resource (Andola, Gairola, & Bhatt, 2012; Kar, Dey, Misra, Chaudhuri, & Sen, 2016; Bhatt et al., 2017). Among others, the berries of *Berberis* species has been investigated for polyphenolics, alkaloids, vitamins, minerals, and other secondary metabolites (Andola, Rawal, & Bhatt, 2011; Belwal, Dhyani, Bhatt, Rawal, & Pande, 2016; Bhatt et al., 2017), which showed nutritional and antioxidant potential (Andola et al., 2012; Negi & Subramani, 2015). The dried fruit juice of *Berberis* has clinically tested and found effective against inflamed acne lesions (Johnson & Rafikhah, 2014), activate immune system and help in prevention of scurvy (Javadzadeh & Fallah, 2012). The fruit extract has been reported for its beneficial effect on cardiovascular and nervous system and can be used for the treatment of hypertension, tachycardia, epilepsy and convulsions (Fatehi-Hassanabad, Jafarzadeh, Tarhini, & Fatehi, 2005; Fatehi et al., 2005). Various food products such as juices, jams, pickles, syrups and candy have been prepared (Javadzadeh & Fallah, 2012) and thus has potential as being used as nutraceutical product (Andola et al., 2012; Bhatt et al., 2017). The formulation and development of products based on plants require deep knowledge on tissue type and process variables to meet the right quality and quantity of the products.

Extraction is an important process and can be designed for obtaining the desired quality and quantity of contents (Belwal, Bhatt, Rawal, & Pande, 2017). A number of new advanced extraction technologies have been evolved and microwave assisted extraction (MAE) is one such technique, which is known for lesser extraction time and solvent consumption, better yield and environmental friendly nature; thus secured its place over conventional extraction techniques (Yuan, Fu, Gu, Zhang, & Fu, 2014; Vázquez et al., 2014; Dahmoune, Nayak, Moussi, Remini, & Madani, 2015). Therefore, microwave is considered as green extraction technology and widely used in improved extraction of compounds from plant material (Li, Fabiano-Tixier, Tomao, Cravotto, & Chemat, 2013a). It works on the principal of dipole rotation and ionic conductance and provides excessive heat in shorter time by the conversion of absorbed energy into heat which depends on solvent dissipation factor (tan δ) (Veggi, Martinez, & Meireles, 2013). Depending on the tissue type, composition and physio-chemical properties of analyte of interest, various factors need to be considered and optimized for better recovery under MAE. As such, microwave power, irradiation time, vessel pressure, solvent type, concentration, pH, particle size and sample to solvent ratio are considered to be important variables in designing the MAE of polyphenolics. Considering the effect of individual variable and optimize using simple univariate methodology (one factor at a time) has many limitations over multivariate (Ebrahimi-Najafabadi, Leardi, & Jalali-Heravi, 2014), and thus Response Surface Methodology (RSM), a multivariate analysis has been used. RSM was found effective not only in sorting out the large number of experimental runs, but also finding model fitness with linear, quadratic and interactive effects (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

This study was designed for the first time to use and optimize MAE conditions for better recovery of nutraceutically important polyphenolics and antioxidants from *Berberis jaeschkeana* fruits and compared the same with ultrasonic (UAE) and maceration extraction (ME) techniques.

2. Materials and methods

2.1. Plant material

Fruits of *Berberis jaeschkeana* was collected from Kedarnath area, Garhwal Himalaya, Uttarakhand (Altitude 3500 m asl), India. These fruits were brought to the analytical laboratory and dried in shade at room temperature (23 ± 2 °C). Fruits showed any sign of deterioration or damaged caused by microbial attack were removed. The seeds were separated from the fruits and the pulp portion was further dried at 37 °C for 3 days. Dried pulp portion was grind to powder using hammer mill (Model-WGM 197, UTS Sales, Delhi, India) with a mesh size of <85 μm and packed in polyethylene bag and stored in refrigerator at 4 °C. All the extracts were prepared within 2 days of storage.

2.2. Chemicals and reagents

Sodium carbonate, potassium persulfate, ferric chloride, sodium acetate, potassium acetate, aluminum chloride, disodium hydrogen phosphate, sodium chloride, hydrogen peroxide, potassium dihydrogen phosphate and hydrochloric acid were purchased from Qualigens (Mumbai, India), and 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), orthophosphoric acid, Folin-Ciocaltue's reagent and methanol from Merck KGaA (Darmstadt, Germany). 2,2-Diphenyl-1-picryhydrazyl (DPPH), ascorbic acid, tannic acid, Folin-Denis reagent and all phenolic standard compounds (rutin hydrate, phloridzin dihydrate, *p*-coumaric acid, (+)-catechin hydrate, gallic acid, quercetin dihydrate, 3-hydroxybenzoic acid, 4-hydroxybezoic acid, ellagic acid, vanillic acid, caffeic acid, *m*-coumaric acid, ferulic acid, *trans*-cinnamic acid and chlorogenic acid) were procured from Sigma-Aldrich (St. Louis, Missouri, United States). All chemicals were of analytical and HPLC grade and the solutions were prepared with methanol and labpure ultra-pure water (Rions India Lab Water Systems, India)

2.3. Microwave assisted extraction and phytochemical analysis

2.3.1. Microwave assisted extraction (MAE)

MAE was carried out using multiwave-3000 microwave reaction system (Anton-Paar, Germany, GmbH). An 8 vessel closed extraction chamber equipped with infrared sensor, pressure and temperature (P/T) sensor, vessel mark sensor and a magnetic stirrer at the base was used with an exhaust outlet at the back of the instrument (Fig. 1). Parameters such as extraction vessel pressure limit (20 psi), oscillation (ON), and IR temperature limit (180 °C) were fixed, whereas microwave power, irradiation time, solvent composition (concentration, volume and HCl concentration) were changed as per the experimental design. Methanol was used as a solvent of choice for its higher dissipation factor (tan δ) under microwaves (Veggi et al., 2013) and further experimental runs were designed for PBD and CCD model as per Design Expert v. 10.0 software (State-Ease, Inc., MN, USA). After extraction, the extract was filtered using Whatman filter paper (No. 1) and the filtrate was stored in a refrigerator at −20 °C during the experiments. All response variables – total phenolics (TP), total flavonoids (TF), total tannins (TT), *in vitro* antioxidant activities (ABTS, FRAP and DPPH) and polyphenolic analysis using HPLC were conducted in triplicates within two weeks and the results expressed as mean values ± standard error.

2.3.2. Determination of total phenolics (TP)

Total phenolic content was measured by Folin-Ciocaltue's colorimetric method (Singleton & Rossi, 1965). TP content was quan-