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Microwave-assisted extraction (MAE) conditions using polynomial design for improving antioxidant phytochemicals in *Berberis asiatica* Roxb. ex DC. leaves

Tarun Belwal[a], Indra D. Bhatt[a,*], Ranbeer S. Rawal[a], Veena Pande[b]

[a] G.B. Pant National Institute of Himalayan Environment and Sustainable Development, Kosi Katarmal, Almora, Uttarakhand, India
[b] Department of Biotechnology, Kumaun University, Nainital, India

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ABSTRACT

Microwave-assisted extraction condition is of great importance for increasing recovery of phytochemicals with lesser time and solvent consumption. In this regard, the advanced microwave extraction technique was applied for the first time for improving polyphenolic antioxidants in *Berberis asiatica* leaves using polynomial design. A combination of first and second order polynomial designs was used at different levels using Plackett-Burman and Box-Behnken design and a total of 29 experiments were conducted for determination of optimal extraction condition for polyphenolics. Under optimal extraction condition [microwave power – 500 W, sample to solvent ratio – 1:45 (g/ml) and methanol concentration – 60%], total phenolics (132.63 mg GAE/g), total flavonoids (31.36 mg QE/g), total tannins (145.17 mg TAE/g), ABTS (34.46 mM AAE/g), FRAP (708.07 mM AAE/g) and DPPH (34.12 mM AAE/g) antioxidant activities was recorded near close with predicted values. The extract also showed DNA and erythrocyte damage protective activity and reveals the presence of 12 phenolic compounds during high-performance liquid chromatography among which 4 compounds were reported first time from this species. Chlorogenic acid and catechin were found in higher concentration as compared with other phenolic compounds. The present study provides the potential of harnessing maximum antioxidant phytochemicals from the *Berberis asiatica* leaves using microwave-assisted extraction condition.

1. Introduction

Berberis asiatica Roxb. ex DC. is widely distributed in Indian Himalayan region from 600 to 2700 m asl (Rashmi et al., 2008). The species is commonly used by the local inhabitants (Bisht et al., 2013) and pharmaceutical industries for its beneficial effect as antidiabetic, antilipidemic, hepatoprotective, antioxidants, etc. (Mokhber-Dezfuli et al., 2014). The beneficial effects of the species are largely due to the presence of various known bioactive compounds such as alkaloids, phenolics, tannins, flavonoids, anthocyanins, vitamins and minerals (Andola et al., 2008, 2011a; Koncic et al., 2010; Belwal et al., 2016). Among others, the presence of phenolics and antioxidant activity in *Berberis* species have been reported (Andola et al., 2008; Belwal et al., 2016) but very few studies are available on optimum extraction conditions (Belwal et al., 2016), and quantification of individual phenolic compounds (Koncic et al., 2010; Singh et al., 2014; Belwal et al., 2016). As such, the presence of rutin, vanillic acid, 3-hydroxybenzoic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, catechin and gallic acid has been reported in *B. asiatica* (Belwal et al., 2016; Bhatt et al., 2017).

Phenolics are large group of compound, known for its antioxidant activity through different mechanism such as scavenging free radical, metal chelation, hydrogen donors, modification of gene expressions and signaling pathways (Leopoldini et al., 2011; Carocho and Ferreira, 2013). Considering their effectiveness in the dietary system and health promoting products, the demand of these natural compounds are increasing. For instance, vitamins and minerals cover 85%, antioxidants 10%, and herbal extract 5% of the total nutraceutical market (Ahmad et al., 2011). The quality and quantity

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; PBD, Plackett-Burman design; BBD, Box-Behnken design; RSM, response surface methodology; TPTZ 2, 4, 6-tripyridyl-s-triazine; GAE, gallic acid equivalent; TAE, tannic acid equivalent; QE, quercetin equivalent; AAE, ascorbic acid equivalent; GA, gallic acid; Q, quercetin; HPLC, high-performance liquid chromatography; DAD, diode-array detection; SEM, scanning electron microscopy; DoE, design of experiment; MAE, microwave assisted extraction; DNA, deoxyribonucleic acid; CV, coefficient of variation; W, Watt.

* Corresponding author.
E-mail addresses: id_bhatt@yahoo.com, idbhatt@gbpihed.nic.in (I.D. Bhatt).

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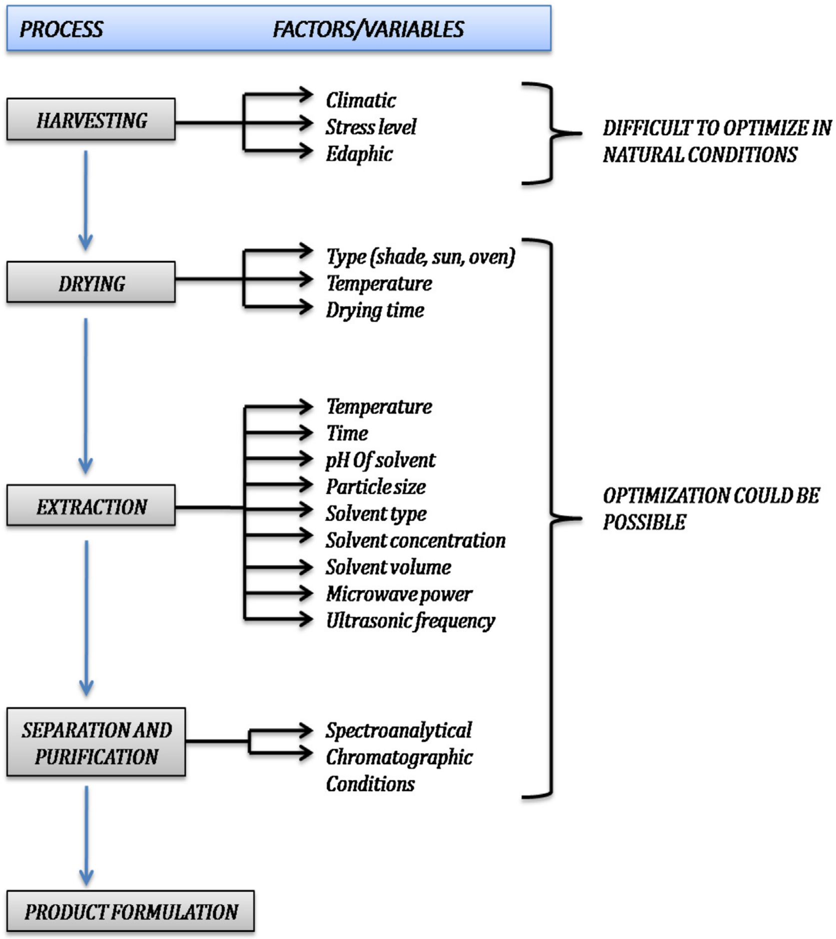

Fig. 1. Different processes and factors involved in herbal/food product formulation and their optimization level.

of phytochemicals present in a medicinal plant depend on environmental conditions, plant growth, harvesting time and various other processing factors (Andola et al., 2011b; Karlund et al., 2014). These include factors such as drying, extraction, separation and purification, which are known to responsible for the quality and quantity of secondary metabolites (Fig. 1). Among these, extraction is a basic and foremost important step in extracting the optimum amount of secondary metabolites. A number of advanced extraction techniques such as ultrasonic-assisted extraction (UAE), microwave assisted extraction (MAE), pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE) are available and proven effective for extraction of phytochemicals (Wang and Weller, 2006; Easmin et al., 2015) but a very few studies are reported on the use of these techniques for extraction of phenolics from *Berberis* leaves (Koncic et al., 2010; Singh et al., 2014). Among others, MAE is known to speed-up the extraction process and better extraction yield along with lesser solvent use as compared with conventional and other advanced extraction techniques (Dahmoune et al., 2015; Nayak et al., 2015). For instance, MAE extraction from *Vaccaria segetalis* Neck. (Yuan et al., 2014), *Eucalyptus robusta* Sm. (Bhuyan et al., 2015), *Citrus sinensis* L. (Nayak et al., 2015), *Achillea millefolium* L. (Milutinovic et al., 2015) and *Cymbopogon martini* Roxb. (Thakker et al., 2016) have proven effective. The effective solvent for MAE depends not only upon the analyte of interest but also on the dissipation factor (tan δ), which measures the polarizability of the solvent under MAE and transformation of absorbing microwaves into heat. To be a good solvent to work under the microwave, the solvent must have high dissipation factor (tan δ). As such, polar solvents such as water, ethanol, methanol and propanol have higher dissipation factor and thus used for MAE over non-polar solvents such as hexane, heptanes, etc. Other than extraction method and type of solvent, the efficient extraction of phytochemicals depends on upon extraction temperature, time, pressure, solvent concentration, solvent volume, pH and particle size. Screening and optimizing these factors using single factor at a time approach at its different levels is a laborious and time consuming; thus possibility of finding any interaction between these factors are suppressed. First and second order polynomial design, when used in combination, can shorten these problems. Plackett-Burman design (PBD) is one such tool, which uses the first order polynomial equation to screen out significant factors, and can be further used for optimization experiments using response surface methodology (RSM).

RSM is an effective mathematical and statistical tool that uses polynomial design for multi-factor studies at a single time (Myers et al., 2016). These designs not only used for lesser experimental runs but also determine interactive effect between the factors. Central composite (CC) and Box-Behnken (BB) designs are commonly used in RSM based upon the need and experimental conditions. As such, extraction method for polyphenols in *Myrtus communis* leaves (Dahmoune et al., 2015), *Achillea millefolium* dust (Milutinovic et al., 2015), *Lycium barbarum* fruits (Mendes et al., 2016), *Berberis asiatica* fruits (Belwal et al., 2016), and *Ocimum basilicum* (Izadiyan and Hemmateenejad, 2016) have been successfully optimized using these techniques.

The present study, therefore, investigated on optimization of MAE conditions for improving phenolics and antioxidant activity in *B. asiatica* leaves. The objectives of the study was to, (a) screen factors affecting MAE of phenolics using first order polynomial

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