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Optimization of ultrasonic-assisted extraction (UAE) of phenolics and antioxidant compounds from rhizomes of *Rheum moorcroftianum* using response surface methodology (RSM)

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ABSTRACT

This study for the first time designed to optimize the extraction of polyphenolic compounds from rhizomes of *Rheum moorcroftianum* using response surface methodology (RSM). Solvent was selected based on the preliminary experiments, and a four-factors-three-level, Box–Behnken Design (BBD) including 29 experimental runs. The polyphenolic content and antioxidant activity was significantly ($p < 0.05$) affected by vessel diameter – 6 cm (X_1), sample to solvent ratio – 1:28.42 g/mL (X_2) and extraction temperature – 37.11 °C (X_3) under ultrasonic assisted extraction (UAE). The measured parameters were found in accordance with the predicted values. High Performance Liquid Chromatography (HPLC) analysis in optimized condition revealed the presence of 12 phenolic antioxidant compounds with the highest concentration of chlorogenic acid (26.68 mg/g). The results indicate that optimization of extraction conditions in *R. moorcroftianum* is critical for precise quantification of antioxidant phenolics and its further utilization in industry.

1. Introduction

The genus *Rheum* consists of approximately sixty species (Rokaya et al., 2012); cultivated for culinary, ornamental and medicinal purposes across the world (Arvindekar and Laddha, 2016), and used in the preparation of jams, jellies and wine (Clementi and Misiti, 2010). In Indian Himalayan Region (IHR), the genus *Rheum* is represented by 10 species and distributed between 2800–4700 m asl (Uniyal et al., 2002; Tabin et al., 2016). *Rheum moorcroftianum* Royle (Family – Polygonaceae), is a Himalayan endemic species commonly known as rhubarb, grows in rocky, bouldery slopes and river banks between an elevation range of 3200–4700 m asl (Uniyal et al., 2002; Rana and Samant, 2010). The genus *Rheum* has been used in the traditional Chinese medicine, Medieval Arabic and Ayurvedic system of medicine (Xiao et al., 1984; Arvindekar and Laddha, 2016). Traditionally, the roots and rhizomes of the *Rheum* are used as an astringent, purgative, tonic and in the healing of ulcers (Anonymous, 2005). Rhubarb contains a variety of bioactive compounds like flavonoids, anthraquinone glycosides, tannins, volatile oils and saponins (Ye et al., 2007; Aslam et al., 2012) and contribute to various pharmacological activities like antifungal, antioxidant, hepatoprotective, nephroprotective and immune modulatory activities (Zargar et al., 2011). Further, anti-cancerous properties of the *Rheum* rhizome in human breast carcinoma (MDAMB-435S) and liver carcinoma (Hep_3B) cell lines is reported (Rajkumar et al., 2011a,b).

The medicinal properties of medicinal plants are attributed to the presence of secondary metabolites, which are unique resources for pharmaceuticals, nutraceuticals, food additives, and fine chemicals (Zhao et al., 2005). These compounds exist in plants and enclosed by insoluble structures such as the vacuoles of plant cells and lipoprotein's bilayers which complicate their extraction process (Corrales et al., 2008). The nature of bioactive compounds and the presence of other biomolecules along with several factors such as extraction methods, type of solvent, pH, temperature, sample-solvent ratio and extraction time are reported to affect yield (Cacace and Mazza, 2003; Chirinos et al., 2007; Ng et al., 2012; Belwal et al., 2017b). However, there is no universally standardized set of optimum conditions for the extraction of bioactive compounds from different plants (Chirinos et al., 2007; Chen

Abbreviations: ABTS, 2, 2-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid); AAE, Ascorbic acid equivalent; BBD, Box–Behnken Design; CV, Coefficient of variation; DPPH, 2, 2-Diphenyl-1-picryhydrazyl; FRAP, Ferric reducing antioxidant power; GA, Gallic acid; GAE, Gallic acid equivalent; HPLC, High performance liquid chromatography; Q, Quercetin; QE, Quercetin equivalent; RSM, Response surface methodology; TAE, Tannic acid equivalent; TFC, Total flavonoid content; TPC, Total phenolic content; TPTZ, 2, 4, 6-Tripyridyl-*s*-triazine; TTC, Total tannin content; UAE, Ultrasonic assisted extraction

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et al., 2007) and Himalayan plants are no exception. Therefore, optimization of species-specific optimal extraction conditions is essential. As such, single factor at a time approach is labor-intensive, time-consuming and do not show any interactive effect. However, the statistical model such as the Response Surface Methodology (RSM) is an effective statistical tool for optimizing complex processes (Zhong and Wang, 2010). RSM is a multi-factors approach with a reduced number of experimental runs, and evaluate multiple parameters and their interactions in a single experiment (Giovanni, 1983; Zhong and Wang, 2010; Myers et al., 2016). It is widely used for optimizing the extraction of polysaccharides, anthocyanins, vitamin E, phenolic compounds, protein and polyphenolics from different plant materials (Cacace and Mazza, 2003; Chandrika and Fereidoon, 2005; Ge et al., 2002; Lee et al., 2005; Li and Fu, 2005; Liyana-Pathirana and Shahidi, 2005; Qiao et al., 2009; Zhong and Wang, 2010; Li et al., 2012, Alberti et al., 2014; Ilaiyaraja et al., 2015; Belwal et al., 2016, 2017a,b). The Box–Behnken design (BBD) is easy to perform experiments and interpret in comparison to other models (Box and Behnken, 1960; Ferreira et al., 2007).

In recent times, the interest of consumers are growing towards sustainability of extracting compounds in nutraceutical industries. This include environmental friendly and low-cost raw materials and technologies (Corrales et al., 2008; Belwal et al., 2018). The traditional extraction methods such as soxhlet, maceration, percolation, etc. are reported to consume large amounts of solvents, time and energy (De Castro and Garcıa-Ayuso, 1998), however, the new extraction technologies showed the higher recovery of valuable compounds in a lesser solvents, lower extraction time and temperature (Barba et al., 2015; Deng et al., 2015; Bouras et al., 2015). Ultrasonic-assisted-extraction (UAE) method is reported to minimize extraction yield through mass transfer phenomena in a number of plant extracts (Corrales et al., 2008; Vilkhu et al., 2008). UAE increased mass transfer rate by cavitation forces, where bubbles in the liquid/solid extraction can explosively collapse and generate localized pressure causing plant tissue rupture and improves the release of intracellular substances into the solvent (Knorr et al., 2004). The feasibility of UAE for the improved extraction of secondary metabolites compared with traditional methods have been highlighted in many research (Mason and Zhao, 1994; Albu et al., 2004; Jian-Bing et al., 2006; Corrales et al., 2008; Belwal et al., 2018) and reviews (Vinatoru, 2001; Knorr et al., 2004; Zhang et al., 2003). Among others, polyphenolics have been categorized under nutraceutically active compound and their role has been justified in treating various ailments. Thus, the present study was designed to determine the optimum UAE condition for maximizing polyphenolic antioxidant extraction yield from *R. moorcroftianum* rhizome.

2. Material and methods

2.1. Plant material

The rhizome portions of *R. moorcroftianum* were collected from five different plants growing near Parvati Lake, Jeolingkong (4500 m asl), Byans valley, Uttarakhand, India. The rhizomes were brought to the plant analytical laboratory of G. B. Pant National Institute of Himalayan Environment and Sustainable Development, Almora India and dried in shade at room temperature. Dried rhizomes were grounded into fine powder using hammer mill (Model-WGM 197, UTS sales, Delhi, India). The grounded powder was passed through a standard mesh size of < 85 μm and stored in airtight bags at 4 °C, until further experimental use.

2.2. Chemicals and phenolic standards

The analytical grade chemicals like 2,2-azinobis (3-ethylbenzthiazoline- 6-sulphonic acid) (ABTS), 2,4,6-tripyridyl-*s*-triazine (TPTZ), acetone, ethanol methanol and propanol were procured from Merck KGaA (Darmstadt, Germany). Ascorbic acid, 2,2-Diphenyl-1-picryhydrazyl (DPPH) radical, and HPLC grade phenolic standards: rutin hydrate, phloridzindihydrate, *p*-coumaric acid, catechin hydrate, gallic acid, quercetindihydrate, 3-hydroxybenzoic acid, vanillic, caffeic, and chlorogenic acid were procured from Sigma-Aldrich (St. Louis, Missouri, United States).

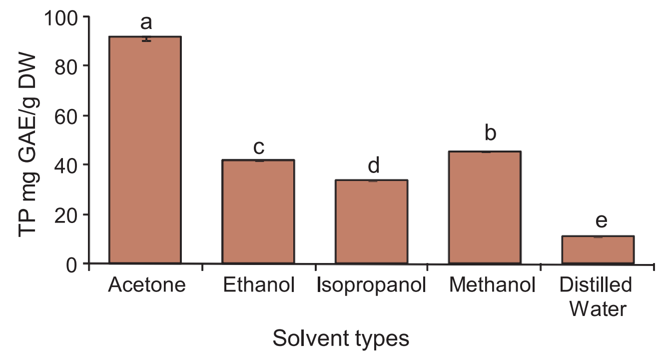

Fig. 1. Effect of different solvents on total polyphenolic content (TPC). Bars capped with same letters are not significantly ($p < 0.05$) different to each other.

2.3. Selection of variables

The effects of different variables such as solvents type and concentration, extraction time, temperature, sample-to-solvent ratio, diameter and shape of the extraction vessel, etc. are known to affect extraction yield and phytochemical contents (Dai and Mumper, 2010). In this context, different solvents such as acetone, ethanol, methanol, isopropanol, and distilled water were tested in a preliminary experiment for selecting suitable solvent (Fig. 1). For this, 2 g sample was extracted in flat based 4 cm diameter vessel (Borosil, India) with 20 mL of solvent (0.2 N) having concentration of 80% and subjected to ultrasonication (50 KHz) using an ultrasonic bath (Toshiba, India) under a temperature of 50 °C for 15 min. Further, similar conditions were applied for the optimization of solvent concentration. All the experiments for each solvent type and concentrations were carried out in triplicates. Selection of best solvent and solvent concentration was based on the maximum value of total phenolic content (TPC).

2.4. Experimental design

Optimization experiment was carried out using response surface methodology (RSM) for extraction of polyphenols from *R. moorcroftianum* rhizomes. Using RSM one can predict the linear, quadratic and interactive effect between the factors *w.r.t.* the responses of a limited number of experiments. A three level, four-factor Box–Behnken design (BBD) (Design Expert trial version 9.0 Stat-Ease Inc., Minneapolis, MN) was applied for the design of experiments, model building and data interpretation. For BBD model, factor levels were varied over 3 levels, and lesser experimental work is required as compared to other models such as central composite design (CCD). In addition, BBD experimental runs allow testing factors to at least one at the center point (0) (Box and Behnken, 1960).

Variables such as vessel diameter (X_1), sample to solvent ratio (X_2), extraction temperature (X_3), and sonication time (X_4) were selected. Keeping the independent variables at three levels (−1, 0, +1), a total of 29 experimental runs were conducted to determine the TPC, TFC, TTC and *in vitro* antioxidant activity (ABTS and DPPH) (Table 1). Further, based on the BBD data, the selection of optimum condition and its validation has been performed (Section 2.8). For each response variables, second order polynomial equation was determined as-