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Polyphenolics in leaves of *Paris polyphylla*: An important high value Himalayan medicinal herb

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ABSTRACT

Paris polyphylla, a source of saponins has not been much studied for polyphenolics and its antioxidant potential. In this investigation, optimization of extraction conditions for polyphenolics compounds using Response Surface Methodology (RSM) in *P. polyphylla* leaf samples was conducted for the first time to look for alternate and renewable source of such compounds. A total of 32 experiments were conducted for screening of variables (*i.e.* ethanol concentration, sample-to-solvent ratio, HCl concentration, extraction temperature and time) using Plackett-Burman and central composite design. Among all the tested independent factors, ethanol concentration (X_1), sample-to-solvent ratio (X_2), and extraction temperature (X_3) showed significant variations in polyphenolic content total phenol (TP), total flavonoids (TF) and total tannin (TT) and *in vitro* antioxidant activity (ABTS, DPPH and FRAP). All the response models showed significant model fitness and a non-significant lack of fit. Under optimal extraction condition, which includes 60% ethanol, 1:40 sample-to-solvent ratio, 45 °C of extraction temperature for 45 min, maximum content of polyphenolics, namely TP (5.36 mg GAE/g dw), TF (44.27 mg QE/g dw), TT (65.41 mg TAE/g dw), ABTS (2.28 mM AAE/g dw), DPPH (12.30 mM AAE/g dw) and FRAP (0.31 mM AAE/g dw) were found. Response values were found to be closer to the model predicted value. Further, High Performance Liquid Chromatography (HPLC) analysis of extract obtained under optimal condition, revealed the presence of 5 phenolic compounds. The present study was found useful in exploring *P. polyphylla* species for polyphenolics and antioxidant potential under optimal extraction condition, which can be of use for pharmaceutical and nutraceutical industries.

1. Introduction

Polyphenols, a diverse and unique group of phytochemicals present in various plants, fruits and vegetables, are being utilized worldwide. The wider health benefits made them suitable for health promotion and prevention of degenerative diseases such as cancer, diabetes, osteoporosis, cardiovascular and neurodegenerative disorders (Fraga et al., 2010; Manach et al., 2004) and thus, accounts for higher demand mainly by nutraceutical and pharmaceutical industries (Lamien-Meda et al., 2008). According to global polyphenols market analysis, it is expected that polyphenolic demand will increase to 33.88 kt by 2024 from 16.38 kt in 2015 (Global Food Antioxidants Market, 2016). The basic mechanism of action of polyphenolics compounds relies on their antioxidant potential by scavenging reactive oxygen species (ROS) and modulating antioxidant enzyme activity (Gechev et al., 2006). They also possess antigenotoxic and antihemolytic activity as shown in various studies (Ashadevi et al., 2014; Belwal et al., 2017b; Kumarappan et al., 2012). Indian Himalayan Region (IHR) holds a large diversity of plants, which have been examined for secondary metabolites and biological activity. Among many, *Paris polyphylla* Smith var. *polyphylla* (Family Melanthiaceae), a perennial herbaceous plant widely distributed in the west Himalaya at an altitudinal range of 1800–3300 m (IUCN, 2004) has drawn much attention in recent years as a source of bioactive compounds. This species contains a large number of bioactive compounds such as steroidal saponins, polyphyllin D, dioscin and balanitin 7 (Deng et al., 1999; Gao et al., 2011; Li et al., 2001; Yuen-Nei Cheung et al., 2005), which found wide application as natural surfactant, food preservative (Cheok et al., 2014) and as antioxidant (Xiao

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; HCL, hydrochloric acid; PBD, Plackett-Burman design; CCD, central composite design; RSM, response surface methodology; ROS, reactive oxygen species; GAE, gallic acid equivalent; TAE, tannic acid equivalent; QE, quercetin equivalent; AAE, ascorbic acid equivalent; HPLC, high-performance liquid chromatography; PDA, photo-diode array; IHR, Indian Himalayan region

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Table 1
List of some species of medicinal plants of IHR investigated for polyphenolics, besides other major active compounds.

Species	Major active compounds	Polyphenolic antioxidants	Reference
Berberis asiatica	Berberine	Rutin, caffeic acid, vanillic acid *etc*	Belwal et al. (2016)
Berberis aristata	Berberine	Total Phenolics	Surveswaran et al. (2007)
Valeriana jatamansi	Valeric acid	Gallic acid, catechin, coumaric acid *etc*	Bhatt et al. (2012), Jugran et al. (2013)
Hedychium spicatum	Hedychenone	Gallic acid	Joshi et al. (2008), Rawat et al. (2011b)
Acorus calamus	α and β-asarone	Total phenolics	Phongpaichit et al. (2005), Raina et al. (2003)
Ginkgo biloba	Ginkgolides	Gallic acid, quercetin	Sati et al. (2013)
Podophyllum hexandrum	Podophyllotoxin	Quercetin	Chawla et al. (2005)
Centella asiatica	Asiaticoside, Madecassic acid, Asiatia acid	Quercetin, catechin	Inamdar et al. (1996), Zainol et al. (2003)
Paris polyphylla	Steroidal saponins	DPPH, Superoxide radicals	Shen et al. (2014)

IHR = Indian Himalayan Region, DPPH = 2, 2-diphenyl-1-picryhydrazyl.

et al., 2014). Moreover, the whole plant extract of *P. polyphylla* have been tested and proven to be effective as antitumor (Lee et al., 2005; Man et al., 2009; Sun et al., 2007; Wu et al., 2004), antifungal (Deng et al., 2008), antihelmintic (Devkota et al., 2007; Wang et al., 2010), antimutagenic (Lee and Lin, 1988) and in the treatment of urinary tract infection (Fu et al., 2008). However, polyphenolics compounds and its antioxidant activity in *P. polyphylla* leaves have not been investigated, which can add more metabolic and functional value and be an alternative source of the active compounds. As such, investigations have been carried out for search of polyphenols and its antioxidant potential in addition to its known active compounds, in some of the medicinal plants of IHR (Table 1).

Efficient extraction of secondary metabolites from plant tissue needs prior information of target compounds on its physical and chemical properties and also on effect of process factors mainly temperature, pH, sample-to-solvent ratio, particle size, extraction time, *etc*. on its final yield (Belwal et al., 2017a). Several statistical tools are available that are used in optimization of extraction process. Among others, response surface methodology (RSM) is one such model that uses limited number of experiments with consideration of linear, quadratic and interactive effect of individual factor on responses. As such, polyphenolics extraction has been successfully optimized using RSM model from various plants (Belwal et al., 2016, 2017a,b; Celaya et al., 2016; Myers et al., 2016; Mujeeb et al., 2015; Pan et al., 2015; Wani et al., 2015). However, such reports on the presence of polyphenolics in *P. polyphylla* leaves sample and its optimum extraction condition are not available. Thus, the present investigation will look on the estimation of polyphenolics and antioxidant potential under optimum extraction condition from *P. polyphylla* leaves, which will surely add more knowledge to its metabolic profile and functional value. Moreover, it can provide an alternative to industries beside its active metabolites.

2. Materials and methods

2.1. Plant samples

Rhizomes of *Paris polyphylla* Smith var. *polyphylla* were collected from wild population of Pindar Valley (30°6′–30°15′N; 78°55′–80°5′E), District Bageshwar, Uttarakhand, India ranging from 1800 to 2700 m. Rhizomes (containing nodes) were brought to the laboratory in perforated polybags sprinkled with 0.2% bavistin (50% carbendazim w/v; BASF India Ltd, Mumbai, India). These were carefully removed from the soil and washed with running tap water for 5 min, excised into various segments (3.0–5.0 cm long pieces), treated with 0.2% bavistin for one hour, washed with distilled water and transplanted into earthen pots (24.0 cm diameter and 20.0 cm height) containing soil and farmyard manure (3:1 w/w) in the Institute greenhouse at Kosi-Katarmal (29°38′15″ N & 79°38′10″ E, 1150 m; District Almora). Watering was done on regular time interval and the plant was found to sprout after 3–4 months of sowing. Leaves (10–12 cm long) were collected after 1–2 months following sprouting and from 10 to 12 plants. The fresh leaves were dried completely in a hot air oven (48 °C ± 1 °C) and ground into powder using Grinder (Mixer-Grinder, Usha, India). The dried powdered samples were packed in a polyethylene bag and kept in refrigerator at (4 °C ± 1 °C) till experimentation.

2.2. Chemicals and reagents

2,2-Diphenyl-1-picryhydrazyl (DPPH) radical, 2,4,6-tripyridyl-s-triazine (TPTZ), hydrochloric acid (HCL) were procured from Hi-media Lab Pvt. Ltd (Mumbai, India). 2,2-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), ascorbic acid and phenolic standards (rutin hydrate, phloridzin dihydrate, *p*-coumaric acid, catechin hydrate, 4-hydroxybenzoic acid, gallic acid, quercetin dihydrate, catechin, caffeic and chlorogenic acid) were procured from Sigma-Aldrich (St. Louis, Missouri, USA) while sodium carbonate, potassium persulphate, ferric chloride, sodium acetate, potassium acetate, aluminium chloride and acetic acid from Qualigens (Mumbai, India). Ethanol and orthophosphoric acid were purchased from Merck (Darmstadt, Germany). All the above chemicals were of analytical or HPLC grade.

2.3. Experimental design (Plackett-Burman and central composite design)

Experimental design for optimization was conducted to screen significant factors using Plackett-Burman design (PBD) and then to optimize their levels using Central Composite Design (CCD) (Fig. 1)

2.4. PBD

A 5 factor 2 level PBD design was selected for screening of significant factors. 12 experimental runs were conducted using ethanol concentration (X_1), sample-to-solvent ratio (X_2), extraction temperature (X_3), HCl concentration (X_4), and extraction time (X_5), as independent variables which vary over two levels (lower and higher) for determining Total Phenolics (TP) as response (Table 2). ANOVA was conducted to find out the significant factors based on their *F-value* at $p < 0.05$. PBD follows first order polynomial equation as-

$$Y = \beta_o + \sum_{i=1} \beta_i X_i \quad (1)$$

where, Y is response time, β_o is a constant, β_i is the linear regression coefficient, X_i is the linear term for ith factor.

2.5. CCD

The significant factors were further tested at three levels *i.e.*, lower (-1), medium (0) and higher (1) using CCD. A total of 20 experimental runs were conducted to determine linear, quadratic and interactive effects between independent factors using second order polynomial equation as

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k-1} \beta_{ij} X_i X_j \quad (2)$$