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An assessment of phytochemical constituents and antioxidant potential of *Delphinium malabaricum* (Huth) Munz

[*Delphinium malabaricum* (Huth) Munz fitokimyasal bileşenlerinin ve antioksidan gücünün değerlendirilmesi]

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ABSTRACT

Objective: The aim of the study was to examine the efficiency of different extraction methods for the determination of total phenolics, flavonoids and alkaloid contents contributing to antioxidant capacity of *Delphinium malabaricum*.

Methods: The extracts of different plant parts (roots, stems and leaves) of *Delphinium malabaricum* were prepared in aqueous and various organic solvents and the extracts were evaluated for phenolics, flavonoids and alkaloid contents as the equivalents of gallic acid, rutin, and colchicine; respectively. The antioxidant capacity of the extracts was also assessed by 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity and ferric reducing antioxidant power assays in both fresh and dry plant tissues and the difference in fresh and dry extracts on phytochemical constituents and antioxidant activities were compared.

Results: The aqueous extracts of roots exhibited the highest total phenolic (4.94 mg gallic acid/g fresh weight, 13.4 mg gallic acid/g dry weight) and total alkaloid content (8.05 mg colchicine/g fresh weight, 20.4 mg colchicine/g dry weight) as compared to stem, leaves and other solvent extracts. Whereas, flavonoid contents were found to be highest in the leaf extracts (5.36 mg rutin/g fresh weight, 7.88 mg rutin/g dry weight). Interestingly the aqueous extracts of all the plant parts exhibited highest yield of phenolic, flavonoids and alkaloids as compare to the other solvents used for the extraction. Antioxidant activity assays exhibited considerable antioxidant potential and showed expected significant positive correlation with the phytochemical compounds.

Conclusion: The study specified that aqueous extracts are more effective to extract phenols, flavonoids, alkaloids and antioxidants from *Delphinium malabaricum* than organic extracts and roots have higher level and the alkaloids were found to be higher comparing to that of phenolics and flavonoids content per gram dry weight of plant tissue.

Key Words: *Delphinium malabaricum*, Phenolics, Flavonoids, Alkaloids, Free radicals, Antioxidant activity

Conflict of Interest: Authors have no conflict of interest.

ÖZET

Amaç: Bu çalışmanın amacı, *Delphinium malabaricum*'un antioksidan kapasitesine katkıda bulunan total fenol, flavonoid ve alkaloid içeriklerin belirlenmesinde farklı ekstraksiyon metodlarının veriminin incelenmesidir.

Metod: *Delphinium malabaricum*'un bitkisinin değişik bölgelerinden (kökler, gövde ve yapraklar) suda ve farklı organik çözücülerde hazırlanan ekstrelerin, fenol, flavonoid ve alkaloid içerikleri sırasıyla gallik asit, rutin ve kolçisin eşdeğeri olarak değerlendirildi. Ayrıca hem taze hem de kuru bitki ekstrelerinin total antioksidan kapasitesi 1,1-difenil-2-pikrilhidrazil serbest radikal süpürme aktivitesi ve ferrik indirgen antioksidan güç testleri ile değerlendirildi. Taze ve kuru ekstrelerin fitokimyasal bileşenleri ve antioksidan aktiviteleri arasındaki fark karşılaştırıldı.

Bulgular: En yüksek total fenol (4.94 mg gallik asit/g taze ağırlık, 13.4 mg gallik asit/g kuru ağırlık) ve total alkaloid (8.05 mg kolçisin/g taze ağırlık, 20.4 mg kolçisin/g kuru ağırlık) içeriği, gövde, yapraklar ve diğer çözücü ekstreleri ile karşılaştırıldığında köklerin sulu ekstresinde bulundu. Bunun yanında, yaprak ekstrelerinde en yüksek flavonoid (5.36 mg rutin/g taze ağırlık, 7.88 mg rutin/g kuru ağırlık) içerik gözlendi. Şaşırtıcı olarak, bitkinin tüm bölgerinden hazırlanan sulu ekstreler, ekstraksiyon için kullanılan diğer çözücüler ile karşılaştırıldığında en yüksek fenol, flavonoid ve alkaloid ürünü ortaya koydu. Antioksidan aktivite deneyleri, dikkate alınacak bir antioksidan aktivite gücü ve fitokimyasal bileşenler ile beklenen pozitif korelasyonu gösterdi.

Sonuç: Bu çalışma *Delphinium malabaricum*'dan fenolleri, flavonoidleri, alkaloidleri ve antioksidanları elde etmek için sulu ekstrelerin organik ekstrelerden daha etkili olduğunu ve köklerin daha yüksek düzeyde ve bitki dokusunda alkoloidlerin, fenoller ve flavonoid içerik ile karşılaştırıldığında gram kuru ağırlık başına daha fazla bulunduğunu belirtmektedir.

Anahtar Kelimeler: *Delphinium malabaricum*, fenol, flavonoid, alkoloid, serbest radikaller, antioksidan aktivite

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Free radicals cause oxidative damage to lipids, proteins and nucleic acids, where active oxygen species such as O_2, OH, or lipid peroxyl radical (LOO) may lead to many biological complications including carcinogenesis, mutagenesis, aging and atherosclerosis [1]. These free radicals are generally mobbed out of the circulation by the various forms of antioxidants. Phytochemical antioxidants are very efficient scavengers of free radicals. The screening of number of plants for their component phytochemicals revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, sterols, terpenes, carbohydrates, lactones, proteins, amino acids, glycosides and saponins [2]. Many of these phytochemicals may help to protect cells against oxidative damage caused by free radicals, which produce a definite physiological action on the human body [3].

Plant species of the genus *Delphinium* are recognized as rich source of biologically active and structurally complex diterpenoid and norditerpenoid alkaloids [4, 5, 6, 7]. Plants bearing norditerpenoid alkaloids are reported to be used as cardiotonic, sedatives, febrifuges and analgesic [8]. Some of these alkaloids possess antifungal activities against human and plant pathogenic fungi, and antifeedant properties to insects *Spodoptera littoralis* and *Leptinotarsa decemlineata* [9]. Majority of the species belonging to *Delphinium* genus are used in herbal folk medicine for the treatment of various diseases. Some of the species are reported to be used as insecticides, antirheumatic and for the treatment of Sciatica [10]. The medicinal uses of *Delphinium* spread over many centuries. Indian species of *Delphinium* find uses in indigenous medicines for destroying maggots in wounds, particularly in sheeps. Pastes and extracts of flowers and roots of many species of this genus have been used traditionally as insecticides, as ingredients of drugs for dysentery and diarrhoea, as tonics for toothache, as cardiac and respiratory depressants and also as a stimulant [11, 12]. Biomolecules from *D. denudatum* are also being studied as potential cures for human diseases. Aqueous extracts from roots have been shown to exhibit properties of antiepileptic drugs [13, 14] and antihepatotoxicity [15]. *D. denudatum* extracts have also been tried in morphine de-addiction [16]. The Himalayan species, *D. brunonianum*, *D. coeruleum*, *D. elatum* and *D. vestitum*, act as cardiac and respiratory depressants [17, 18]. Some of the *Delphinium* species also had shown insect repellent, antioxidant, anti inflammatory and tyrosinase inhibition activities [19]. *Delphinium malabaricum* (Huth) Munz., one of the endemic species of the genus *Delphinium*, found in the Western Ghats of Maharashtra [20] has not been investigated for its medicinal potential. Therefore, it is interesting to study the ability of *Delphinium malabaricum* phytochemical components contributing to its antioxidant activity. The objective of this research is to study the possibility of using *Delphinium malabaricum* as an antioxidant by examining its ability to scavenge free radicals.

Materials and Methods

Plant material and reagents

The plant material viz., roots, stems and leaves of *Delphinium malabaricum* were collected from the experimental plots within the Botanical garden of Botany Department, Shivaji University, Kolhapur. All the chemicals and solvents used for assays were of analytical grade. 1,1-diphenyl-2-picrylhydrazyl (DPPH·, 95%), 2,4,6-Tri (2-pyridyl)-s-triazine (TPTZ) and all the standards were procured from Sigma-Aldrich Mumbai, India.

Preparation of extract

Variation in extraction methods are usually depend on the length of the extraction period, solvent used, pH of the solvent, temperature, particle size of the plant tissues and the solvent-to-sample ratio. The basic principle is to grind the plant material (fresh or dry) finer, which increases the surface area for extraction thereby increasing the rate of extraction. In this study the extraction of plant material was achieved by homogenizing the fresh and dry plant tissue in various solvents. Earlier studies reported that solvent-to-sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal [21]. The extraction method that has been widely used by researchers is plant tissue homogenization in solvent [22]. Dried or wet, fresh plant parts are ground in a blender to fine particles, put in a 5 ml of solvent and shaken vigorously for 5 - 10 min and left for 24 h in a shaking machine after which the extract is filtered. Then the extracts obtained were centrifuged at 8,000 xg for 15 minutes. The supernatant was collected and the residue was again suspended by adding 5 ml of solvents and centrifuged to complete the extraction. The supernatants pooled and the volume was adjusted to 10 ml by dilution of more distilled water. Same procedure was followed for the preparation of other solvent extracts (methanol, ethanol and acetone). All the extracts were kept at 4oC and for the assays 1% (v/v) extracts (diluted with double distilled water or respective solvents) were used.

Determination of total phenolic content

Total phenolic contents (TPC) of the plant extracts were determined using Folin-Ciocalteu method [23]. The reaction mixture was prepared by mixing an aliquot of the extracts (0.125ml) with Folin-Ciocalteu reagent (0.125ml) and 1.25 ml of saturated Na_2CO_3 solution. Reaction mixture was thereafter incubated for 90 min at room temperature. The absorbance was measured at 760 nm. The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained. A calibration curve was prepared, using a standard solution of gallic acid (10 µg/ml to 100 µg/ml, $r^2 = 0.993$). Results were expressed in terms of mg gallic acid equivalents (GAE)/ g fresh weight (fw) or dry weight (dw) of sample.

Determination of total flavonoid content

Total flavonoid contents (TFC) of the plant extracts were analyzed according to the spectrophotometric method [24]. The reaction mixture was prepared by adding 1.5 ml of extract to 1.5 ml of 2% methanolic $AlCl_3$. Samples were incubated for 10 min at room temperature. The absorbance was measured at 368 nm. The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained. The same procedure was used for the standard solution of rutin and the calibration curve was prepared using a standard solution of rutin (10 µg/ml to 100 µg/ml, $r^2 = 0.964$). The results were expressed on a fresh weight (fw)/dry weight (dw) basis as mg rutin equivalents (RE)/ g of sample.

Determination of total alkaloid content

Total alkaloid content (TAC) of the extracts was measured using 1,10-phenanthroline method described by Singh *et al*., [25]. The assay mixture was prepared and incubated for 30 minutes in water bath maintained at 70 ± 2oC. Above reaction mixture excluding 1% plant extract, substituted by distilled water served as a blank. The absorbance was measured at 510 nm against reagent blank. The absorbance measurements were compared to standard curve of colchicine (a standard alkaloid) and expressed as milligrams of colchicine equivalents (CE) per gram of fresh weight (fw)/dry weight (dw) of respective plant parts of Delphinium malabaricum.

Screening of alkaloids by thin layer chromatography

Five g of the dried sample (Rhizome) was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide (12.5%) was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide (1%) and then filtered. The residue is the alkaloid, which was dried and weighed [26]. The amount of alkaloid was calculated using the formula.

Total Alkaloids (%) = The weight of alkaloid residue (X)/ Weight of sample (W) X100

X – Weight of the residue

Y – Weight of the filter paper

Z – Weight of the filter paper + alkaloid residue

Total (X) = Z- Y

Thus the crude extract of alkaloids obtained was weighed and dissolved in a known volume of ethanol and was used for TLC. For this technique precoated TLC aluminium sheets (20 x 20 cm Silica gel 60 F_{254}) were used. The alkaloid spots were separated using the solvent mixture chloroform and methanol (15:1). The color and Rf values of the separated alkaloids and the reference compound colchicine were recorded both under ultraviolet (UV 254 nm) and visible light after spraying with Dragendorff's reagent.

DPPH free radical-scavenging assay

The ability of the plant extracts to scavenge DPPH free radical (1,1-diphenyl-2-picrylhydrazyl) was assessed by using the method of Aquino *et al.*, [27]. Plant extract (25µl) was mixed with 3ml of 25mM DPPH solution. After 20 min incubation in the darkness at room temperature, the absorbance was measured at 515 nm, against a blank of ethanol without DPPH. Results were expressed as percentage of inhibition of the DPPH radical and were calculated by the following formula.

Scavenging activity (%) = [1-(Absorbance of sample/Absorbance of control)] X 100, where Absorbance of control is the absorbance of DPPH solution without extracts.

Ferric reducing antioxidant power assay (FRAP assay)

The ability to reduce ferric ions was measured using a modified version of the method described by Pulido *et al.*, [28]. An aliquot (90µl) of extract was added to 2.7 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM TPTZ solution and 1 part of 20 mM $FeCl_3$. $6H_2O$ solution) and the reaction mixture was incubated at 37°C for 15 min. After that, the absorbance was measured at 593 nm. A calibration curve was prepared, using an aqueous solution of ascorbic acid (100 µM to 1000 µM, $r^2 = 0.998$). FRAP values were expressed on a fresh weight (fw)/dry weight (dw) basis as micromoles of ascorbic acid equivalent per gram of sample.

Statistical analysis

All the experiments were carried out in triplicate and the measurements were expressed as average results of three analyses±standard deviation (SD). The magnitude of correlation between variables was done using Graphpad Prism Software program-version 3 [29].

Results

Phytochemical analysis

The assessment of *Delphinium malabaricum* for phytochemical compounds in different plant parts at both fresh and dry weight basis was presented in Table 1. The total phenolics, flavonoids and alkaloid content of sample extracts obtained from different plant parts of *D. malabaricum* varied with the solvents used for the extraction (Table 1). The concentration of total phenolics in the examined extracts ranged from 0.60 to 13.4 mg GAE/g fw/dw. Among the four different extracting solvents used, the aqueous extracts of roots rendered highest phenolic content (4.94 mg GAE/g fw and 13.4 mg GAE/g dw) followed by methanol extracts (1.62 mg GAE/g fw and 9.48 mg GAE/g dw), ethanol extracts (2.01 mg GAE/g fw and 3.05 mg GAE/g dw) and acetone extracts (1.92 mg GAE/g fw and 2.27 mg GAE/g dw). Overall, the aqueous extracts showed higher phenolic contents than those of other solvent extracts. Similarly, the total flavonoid con-

Table 1. Total phenolics, flavonoids and alkaloid content in different plant parts of *Delphinium malabaricum*

Solvents	Plant material	Total Phenolics (mg GAE/ g fresh/dry weight)			Total Flavonoids (mg RE/g fresh/dry weight)			Total Alkaloids (mg CE/g fresh/dry weight)		
		Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
Aqueous	Fresh	4.94 ± 0.51	1.48 ± 0.04	3.01 ± 0.05	2.22 ± 0.08	0.89 ± 0.07	5.36 ± 0.04	8.05 ± 0.12	7.12 ± 0.28	7.27 ± 0.24
	Dry	13.4 ± 0.72	5.02 ± 0.48	8.08 ± 0.22	6.08 ± 0.70	4.10 ± 0.08	7.88 ± 0.08	20.4 ± 1.51	11.0 ± 0.10	7.75 ± 0.20
Methanol	Fresh	1.62 ± 0.19	0.60 ± 0.04	1.56 ± 0.12	0.21 ± 0.05	0.61 ± 0.16	3.75 ± 0.08	7.42 ± 0.21	5.32 ± 0.24	6.91± 0.09
	Dry	9.48 ± 0.21	2.48 ± 0.15	3.38± 0.43	1.19 ± 0.23	2.09 ± 0.49	7.05 ± 0.36	13.1 ± 0.39	9.53 ± 0.26	8.30 ± 0.31
Ethanol	Fresh	2.01 ± 0.22	1.11 ± 0.26	1.63 ± 0.07	1.28 ± 0.42	0.74 ± 0.03	1.68 ± 0.31	7.33 ± 0.37	5.81 ± 0.16	5.66 ± 0.14
	Dry	3.05 ± 0.21	1.26 ± 0.11	2.15 ± 0.11	1.31 ± 0.02	2.40 ± 0.71	4.68 ± 0.58	9.19 ± 0.20	8.63 ± 0.09	7.66 ± 0.14
Acetone	Fresh	1.92 ± 0.06	0.96 ± 0.08	1.42 ± 0.33	0.62 ± 0.08	0.77 ± 0.07	3.10 ± 0.27	7.02 ± 0.46	5.99 ± 0.09	5.89 ± 0.10
	Dry	2.27 ± 0.31	1.12 ± 0.11	1.49 ± 0.22	0.76 ± 0.06	1.79 ± 0.31	4.62 ± 0.90	10.1 ± 0.13	7.03 ± 0.41	7.72 ± 0.38

Values are expressed as mean±SD of triplicate measurements; mg GAE/ g fresh/dry weight: milligram gallic acid equivalent per gram fresh or dry weight; mg RE/g fresh/dry weight: milligram rutin equivalent per gram fresh or dry weight; mg CE/g fresh/dry weight: milligram colchicine equivalent per gram fresh or dry weight.

tent values were found to be higher in leaf extracts prepared in different solvent systems than those of stem and roots (Table 1). Amongst all the solvents tried, here also the aqueous extraction system for leaves was found to be the most efficient with the extraction of highest flavonoid content at both fresh weight and dry weight bases (5.36 mg RE/g fw and 7.88 mg RE/g dw). However, the total flavonoid contents in the different extracts ranged from 0.21 to 7.88 mg RE/g fw/dw.

The quantification of total alkaloids in the analyzed samples revealed that D. malabaricum was clearly the species presenting significantly higher contents of alkaloids (Table 1). The total alkaloid content (TAC) of the extracts in fresh and dry plant tissue ranged from 7.12 to 20.4 mg CE/g fw/dw for aqueous extracts, while it ranged from 5.32 to 13.1 mg CE/g fw/dw for methanol extracts, 5.66 to 9.19 mg CE/g fw/dw for ethanol extracts and from 5.89 to 10.1 mg CE/g fw/dw for acetone extracts. Overall from these observations it was revealed that the dry plant extracts yields more content than fresh extracts. Fresh plant extracts may contain lower amounts of bioactive principles due to a water content of typically 75 to 95%, resulting in a marked dilution effect [30]. Among the tested plant parts, the highest value of alkaloids was present in root extracts as compared to stem and leaf extracts. Thus the results revealed that the roots of *Delphinium malabaricum* were rich in alkaloids as determined by the method described by Singh *et al.* [25]. Further the analysis of roots for alkaloid content by the method describe by Harborne [26] determined that the root extracts of *D. malabaricum* contained the highest percentage crude yield of alkaloids (3.13% - 31.3 mg/g dry weight). Thus it was found that extraction of alkaloids by Harborne [26] method was most suitable for more yields of total alkaloids. The alkaloids present in the roots are well extracted through ethanol with 10% acetic acid [26].

Since the preliminary phytochemical screening showed the presence of a considerable amount of alkaloids which are the major phyto-constituents, so it is dual necessary to perform thin layer chromatography for their further confirmation. During chromatographic separation, it was observed that alkaloids appeared as orange color bands when sprayed with Dragendorffs reagent (Fig. 1a). The results of the TLC studies demonstrated the presence of 10 different types of alkaloids with 10 different Rf values with range from 0.06 ± 0.005 to 0.61 ± 0.01 indicating the presence of different types of alkaloids in *D. malabaricum*. A blue color band of reference compound colchicine was seen under ultraviolet (UV 254 nm) light with an Rf value of 0.61 ± 0.01. The 10th band of the extract components gave identical Rf value with that of standard alkaloid detected under UV light, which reveals the possibility of the presence of colchicine in the plant material (Fig. 1b). However, the other bands of alkaloids developed on the TLC plate were not identified. The result of the study authenticates and confirms the presence of colchicine and

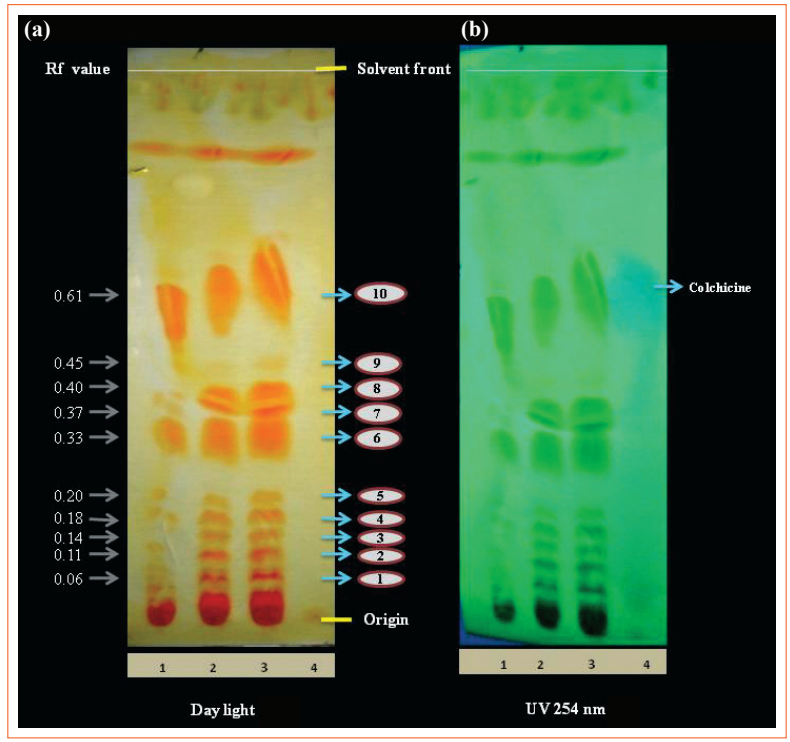

Figure 1. TLC profile of alkaloids extracted from the roots of *Delphinium malabaricum* by Harborne method (1973); the alkaloids were visualized as orange spots after spraying with Dragendorff's reagent. **(a)** TLC plate exposed to day light, **(b)** TLC plate exposed to UV 254 nm. 1-3: Different concentration of extracts (1: 25µl, 2: 50µl, 3: 100µl), 4: Standard alkaloid (Colchicine); the 10th band of the alkaloid has shown similar Rf value with that of authentic colchicine detected under UV illumination at λmax 254 nm.

several other alkaloids in *D. malabaricum*.

Antioxidant capacity

The antioxidant activities of various sample extracts of *Delphinium malabaricum* was determined by DPPH free radical scavenging activity and ferric reducing antioxidant power (FRAP) assays. The experimental results revealed that the extracts of different plant parts prepared in various solvents (i.e. aqueous, methanol, ethanol and acetone) indicated unsteady pattern in scavenging activity of free radicals (Table 2). All the root extracts (prepared in different solvents) possessed higher DPPH radical scavenging activities as compared to stem and leaves. The extraction of antioxidant substances of different chemical structure was achieved by using solvents of different polarity. Results obtained showed that the aqueous extracts of roots contained higher antioxidant activities compared to all the other extracts tried in the experiment. It appears that root extracts have a strong hydrogen-donating capacity and can efficiently scavenge DPPH radicals. Amongst all the plant parts and solvents used for extraction there was very less notable antioxidant activity observed in the stem and leaf extracts. The elevated DPPH radical scavenging ability of the root extracts might be due to the presence of high contents of phenolics or alkaloids.

The results of ferric reducing antioxidant power are in coordination with the DPPH results and indicated that the reductive potential of roots in all four solvents was higher than that in stem and leaf extracts (Table 2). The root extracts showed higher ferric-reducing ability than that of stem and leaf extracts. In this assay, the aqueous extracts again showed relatively high antioxidant activity among all the solvents used for the extraction. The presence of reductants in the plant extracts caused the reduction of the Fe3+-TPTZ complex to the blue ferrous (Fe2+) form. The reducing activity of a compound might serve as a significant indicator of its potential antioxidant activity. There was a significant correlation between DPPH and FRAP assay of root extracts. The higher the DPPH-scavenging activity, the higher the FRAP activity of the samples.

Discussion

Plants were the potent source of phytochemical constituents that are responsible for its pharmacological activities. Many phytochemical compounds were isolated from various plant species such as phenolics, flavonoids, tannins and alkaloids [31]. Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Many authors had reported about plant extract preparation from the fresh plant tissues [32]. The reason behind this came from the ethno medicinal use of

Table 2. Antioxidant capacity in different plant parts of *Delphinium malabaricum*

Solvents	Plant material	DPPH (%) (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity			Ferric reducing antioxidant power (μM AAE /g fresh/ dry weight)		
		Root	Stem	Leaf	Root	Stem	Leaf
Aqueous	Fresh	56.05%	35.99%	36.99%	3547.17 ± 10.1	2087.74 ± 3.81	3126.10± 10.1
	Dry	76.03%	55.10%	63.32%	5919.31 ± 30.3	4183.86 ± 7.68	4276.89± 13.2
Methanol	Fresh	35.66%	34.58%	27.07%	3203.26 ± 6.61	1683.42 ± 4.57	3070.98± 4.40
	Dry	73.97%	50.06%	33.12%	5734.12 ± 13.2	3068.78 ± 14.4	3120.28± 3.81
Ethanol	Fresh	42.36%	30.21%	30.58%	3721.34 ± 7.63	1712.08 ± 3.28	2429.45± 12.2
	Dry	68.22%	49.16%	40.59%	4113.75 ± 6.71	3148.14 ± 6.61	3880.07± 10.1
Acetone	Fresh	37.36%	17.44%	20.17%	3604.49 ± 4.82	2005.73 ± 23.2	3220.89± 6.61
	Dry	70.07%	33.87%	32.79%	5090.38 ± 10.1	2802.02 ± 20.2	3511.09± 8.21

Values are expressed as mean±SD of triplicate measurements; μM AAE /g fresh weight: micro molar ascorbic acid equivalent fresh or dry weight.

fresh plant materials among the traditional and tribal people. But as many plants are used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, plants are usually air dried to a constant weight before extraction. Medicinal plants can be used in fresh or dried form. Drying is the most common method for post-harvest preservation of medicinal plants and must be accomplished as soon as possible after harvesting, to increase the quality of plants and to prevent the expected contamination and losses [33], so the assessment of *D. malabaricum* for phytochemical constituents and antioxidant potential was attained in both fresh and dried plant tissue. The results revealed that the content of phenolics, flavonoids and alkaloids in different plant parts of *D. malabaricum* showed the presence of high phenolics and alkaloid content in root extracts whereas the leaf extracts exhibited the presence of high flavonoid content at both fresh weight and dry weight basis. These results are in agreement with previous works reporting high leaf polyphenol proportions in other plants [34], thus confirming that leaf function serves as defense mechanism against UV damage [31]. Exposure to increased levels of UV radiation causes the leaves to redden and increases the concentrations of total phenols and the main flavonoids [35].

Delphinium species are well known as a source of alkaloids of pharmaceutical importance. Alkaloids, as a rule, do not occur singly in plants. The plant usually produces a series of alkaloids, which may differ only slightly in physical and chemical characters. In the plants, alkaloids may be systemic, i.e. distributed throughout, or restricted to specific organs like roots (Aconite, Belladona), rhizomes and roots (Ipecac, *Hydrastis*), stem barks (*Cinchona*, Pomegranate), leaves (*Hyoscyamus*, Belladona), fruits (Pepper, *Conium*) or seeds (Nuxvomica, *Areca*) [36]. In present study the plant analyzed for their phytochemical compounds had shown highest alkaloid content in root extracts. Further the analysis of alkaloids using thin layer chromatographic technique confirmed the presence of alkaloids which comply with the results of preliminary phytochemical screening. The results of the TLC analysis indicated the presence of colchicine in *D. malabaricum*. TLC is the principle technique of chromatography. Therefore, further analysis by higher techniques such as High performance liquid chromatography (HPLC) or NMR (Nuclear magnetic resonance) spectroscopy for clear identification of alkaloids present in D. malabaricum is desired.

Many Indian medicinal plants are considered potential sources of antioxidant compounds. In some cases, their active constituents are known. *Terminalia chebula*, *T. bellerica*, *T. muelleri*, and *Phyllanthus emblica*, all of which have antioxidant activity, showed high content of phenolics like gallic acid [37, 38]. Flavonoid groups such as rutin and apigenin from *Teucrium polium* L. have antioxidant and free radical scavenging activities [39]. Condensed tannin from *Rosaceae* plant roots [40] and some alkaloid groups such as linearilobin and linearilin [41] from *Delphinium linearilobum* showed free radical scavenging activities, natural products from a variety of plants have been utilized for the treatment of various diseases.

The antioxidant activities of phytochemicals including phenolics, flavonoids and alkaloids have been reported in the present study. The foregoing results of antioxidant profile of *D. malabaricum* specified that the extracts studied can act as electron donors and react with free radicals and convert them to more stable products, thus terminating the radical chain reaction. The comparison of solvent systems used for the extraction reveals that the aqueous extracts were found to have higher free radical scavenging and ferric ion reducing capacity comparable with that of the methanol, ethanol and acetone extracts of respective samples and were also found highest in phenolics, flavonoids and alkaloid content in all the plant parts analyzed, suggested water as most efficient solvent for extraction due to highest yield. This higher yield of water

extract might be because *Delphinium malabaricum* contained more water-soluble substances.

The correlation of the phytocompounds with the antioxidant activity showed both positive and negative correlation. The positive correlation indicated that phytochemicals are the main factors contributing to the antioxidant properties of *Delphinium malabaricum*. Further the negative correlation between TPC, TFC, TAC and antioxidant activity suggested that it could be related to other antioxidant compounds contained in the plant. Thus there are no universal criteria for presence or absence of antioxidant activity in different plants. There are many reports that phenols and antioxidant activity is directly correlated. Phenolic content can be an indicator of the antioxidant capacity of the extract. However the plants are rich in secondary metabolites and it is not known which one predominates and also the mechanism of action of different antioxidant assays is different. In *Delphinium malabaricum* the presence of alkaloids with high concentration showed a good correlation with antioxidant activity. Thus the result indicates that alkaloids are the major contributors of antioxidant capacities of this plant. The pharmacological activities are often attributed to the alkaloids found in the roots. The results strongly suggest that alkaloids are important components of this plant and some of its pharmacological effects could be attributed to the presence of these valuable constituents.

Conclusions

Phytochemical investigation of *Delphinium malabaricum* indicates the presence of phenolics, flavonoids and high concentration of alkaloids in different plant parts. The content of phytochemical constituents and the relative antioxidant activity of dry plant extracts were higher than fresh ones. The determination of antioxidant capacity revealed that greater antioxidant power was may be because of the presence of identified phytochemicals. There was a good correlation between the alkaloid content and antioxidant capacity of the root extracts. Thus the presence of alkaloids with high concentration and its correlation with highest antioxidant capacity in roots indicates that alkaloids are the major contributors of antioxidant capacities in this plant. The plant studied here can be seen as a potential source of useful drugs. Further studies are needed to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

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