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Research Article

Phytochemical Analysis and Antioxidant Potential of *Delphinium denudatum* Wall

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Abstract

Delphinium denudatum Wall is an annual herb known for its medicinal property from the earlier literature. Although many works have attributed to screen the medicinal property of this plant rhizome, but the role of different solvents in extraction and screening of the active ingredients present in the rhizome of the plant remain unexplored. Total rhizome extracts of D. *denudatum* (rhizome) was taken for the present investigation displayed the presence of various bioactive compounds. The bioactive compounds of the selected rhizome have been screened using different solvents such as ethanol, petroleum ether, ethyl acetate and chloroform. From this result, it has concluded that the *D. denudatum* ethanolic extract have shown to possess maximum amount of alkaloids, steroids, terpenoids and flavonoids, where as petroleum ether, ethyl acetate and chloroform are comparatively less. The ethanolic extract of *D. denudatum* was having free radical (DPPH, and SOS) inhibitors acting as primary antioxidants whereas ethyl acetate had hydroxyl radical scavenger as a primary antioxidant than other three extracts.

Keywords**:** *Delphinium denudatum*, Antioxidant, Rhizome, Glycosides and Alkaloid.

INTRODUCTION

Delphinium denudatum Wall belongs to the family – *Ranunculaceae* is found on the outer ranges of Western Himalayas and from Kashmir to Uttrakhand, altitudes of 2400–3650 M. Jadwar, Nirvishi, Nirbisi, Nirbasi, Zadwar, Avisha and Vishalakarani are some of its vernacular names (Murray, 1984). It is one of the most important drugs used in the Unani medicine system of medicine in India (Dymock *et al*., 1990). The rhizome is bitter, stimulant, alterative, tonic, used in a variety of ailments such as aconite poisoning, brain diseases, fungal infection, piles and toothache as analgesic and astringent (Rahman *et al*., 1997; Ahmad *et al*., 1998; Zafar *et al*., 2003 and Raza *et al*., 2003). Only few studies have been reported on the presence of di-terpenoid alkaloids, steroids, fatty acids and sugars (Asif *et al*., 1981; Anonymous, 1992 and Rahman *et al*., 1997). But there are not much reports stating the detailed analysis of the phytochemical and antioxidant properties of rhizome of *Delphinium denudatum*, hence in the present study is focusing on these aspects.

MATERIALS AND METHODS

Phytochemical analysis of *D. denudatum*

Collection of rhizome

Rhizome of *D. denudatum* (Tamil- Nirbasi) was purchased from khari baoli market, Old Delhi, India. The botanical identification was authenticated by Prof. P. Jayaraman, Ph.D, Director, Plant Anatomy Research Center, Chennai.

Plant extract preparation

The rhizome sample of *D. denudatum* was washed, shade dried. Rhizome were chopped into small pieces and ground to a fine powder by using pulverizer. About 50 g of powered rhizome material was packed in the filter paper and placed gently in the Soxhlet apparatus. 300 mL of Ethanol was taken as the extraction solvent. The extraction was carried out for 15 reflexes at 50°C. After 15 reflexes the crude extract was further concentrated by using rotary evaporator and resultant extract was used for the further study. Likewise, petroleum ether extract (PE), ethyl acetate (EAE) and chloroform extract (CE) were extracted and concentrated and resulted extracts were used for the further study.

Qualitative phytochemical screening

The qualitative phytochemical tests were performed for establishing profile of given extract for its chemical composition. Qualitative phytochemical analyses were done using the procedures of Kokate *et al* (1995); Waldi, 1965; Sofowara *et al*., 1993; Sathish *et al*., 2007 and Umesh *et al*., 2010.

Conformation of steroid by UV and TLC analysis

Presence of steroid was analyzed using thin layer chromatography by spraying the TLC plate with methanol H_2SO_4 reagent.

***In vitro* Antioxidant assay**

DPPH radical scavenging assay

The free radical scavenging abilites of the test extracts were determined by using the change in absorbance of DPPH (2, 2-diphenyl-1-picryl-hydrazyl or 1, 1- diphenyl-2-picrylhydrazyl) at 517 nm using the spectrometric method described by Van Acker *et al*., 1996. The reaction mixture comprised of 2 mL of methanol solution of DPPH (0.3 mM) and 1 mL of standard or ethanolic extract (different concentration), dissolved in DMSO. The total reaction volume was 3 mL. The reaction mixture was incubated at 37°C for half an hour in dark. After incubation, decrease in absorbance was measured at 517 nm using UV spectrophotometer (UV-1601 Shimadzu) against control. The control contained 1 mL of DMSO, instead of test sample. DPPH scavenging activity was compared to ascorbic acid.

$$\text{DPPH scavenging effect (\%)} = [(A_1 - A_0)/ A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of sample or standard. The inhibition concentration at 50% inhibition (IC_{50}) was the parameter used to compare the radical scavenging activity.

Superoxide anion radical scavenging assay

The superoxide anion radical scavenging activity of plant extract was determined by method of Salah *et al*., 1995. Various concentrations of plant extracts (0.1 mL) was mixed with 1 ml of nitro blue tetrazolium (NBT) solution in Tris-HCl buffer (16 mM, pH 8) and 1 mL of NADH solution in Tris-HCL buffer. The reaction was started by adding 0.5 mL of phenazine methosulfate (PMS) solution to the mixture, incubated at 25°C for 5 min and absorbance was measured at 560 nm in UV spectrophotometer (UV-1601 Shimadzu) against control samples. The control contained 1 mL of DMSO, instead of test sample. Superoxide anion radical scavenging activity was compared to ascorbic acid.

$$\text{SOS scavenging effect (\%)} = [(A_1 - A_0)/ A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of sample or standard. The inhibition concentration at 50% inhibition (IC_{50}) was the parameter used to compare the radical scavenging activity.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to the methods described by Van Acker *et al*., 1996. 0.1 mL of the various concentration of extract and 1 mL of iron-EDTA solution (0.1% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of DMSO were added. The reaction was initiated by adding 0.5 mL of 0.22% ascorbic acid and heated on a water bath at 80–90°C for 15 min. The reaction was terminated by the addition of 1 mL of ice cold TCA (17.5% w/v) and 3 mL of Nash reagent (75 g of ammonium acetate, 3 mL of glacial acetic acid, and 2 mL of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at room temperature for 15 min for the color development. The absorbance was measured at 412 nm using UV spectrophotometer (UV-1601 Shimadzu) against control samples. Hydroxyl radical scavenging activity was compared to ascorbic acid.

Hydroxyl radical scavenging effect (%) = $[(A_1–A_0)/A_0] \times 100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of sample or standard. The inhibition concentration at 50% inhibition (IC_{50}) was the parameter used to compare the radical scavenging activity.

Estimation of total phenolis

The total phenol content (TPC) was determined by Folin-Ciocalteu reagent method of Salah *et al*., 1995. A volume of 0.5 mL of the extract (different concentration) and 1 mL of Folin-Ciocalteau reagent (0.5 N) were mixed and incubated at room temperature for 15 min. 2.5 mL saturated sodium carbonate was added, incubated in the dark for 30 min at room temperature and absorbance was measured at 760 nm using UV spectrophotometer (UV-1601 Shimadzu). The total phenol content was expressed in terms of gallic acid equivalent (mg/g dry extract).

Estimation of total flavonoids

The total flavonoid content was determined by Aluminum chloride method of Salah *et al*., 1995. The reaction mixture (3 mL) that comprised of 1 mL of plant extract (different concentration) was added to 0.5 mL of sodium nitrate (5%). After 5 min at 25°C, 0.5 mL of aluminum chloride (10%) was added and further 5 min the reaction mixture was treated with 0.2 mL sodium hydroxide (1 nm). Finally the reaction mixture was incubated at room temperature for 30 min and absorbance was read at 510 nm using UV spectrophotometer (UV-1601 Shimadzu). The total flavonoid content was expressed in terms of quercetin equivalent (mg/g dry extract).

RESULTS AND DISCUSSION

Phytochemical analysis showed the presence and absence of certain chemical constituents in four extracts such as ethanol, petroleum ether, ethyl acetate and chloroform extracts. The phytochemical screening of different solvent extracts of the root of *D. denudatum* revealed the presence of medically bioactive constituents, shown in Table 1. Alkaloids, carbohydrate, steroids, phenolic groups, protein, amino acid, terpenoids and flavonoids were present in the ethanolic extract of *D. denudatum* where as saponins, tannins, and glycosides were absent. Steroids, glycosides, terpenoids and flavonoids were found in the petroleum ether extract but alkaloids, amino acid, carbohydrate, saponins, phenolic groups, protein, flavonoids and tannins were absent. In ethyl acetate extract, flavonoids, phenolic groups and tannins were present and alkaloids, terpenoids, steroids, saponins, amino acid, protein, carbohydrate and glycosides were absent. In chloroform extract, alkaloids, steroids, glycosides, terpenoids, phenolic groups, tannins and flavonoids were present except for carbohydrate, protein, saponins, and amino acid. In this study, predominant components such as steroids, alkaloids and terpenoids were present in all the solvent crude extracts of *D. denudatum.*

Plants have rich sources of active metabolites like tannins, alkaloids, flavonoids, phenols, steroids, and volatile oils, which are responsible for their therapeutic activities (Rabe and Vanstoden, 2000). Most natural alkaloids and their derivatives are used as basic medicinal agents for their analgesic and antibacterial effects. Flavonoids are water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage (Salah *et al*., 1995). Atta-ur-Rahman *et al*. (1997) reported that the alkaloids like 8-acetylheterophyllisine, vilmorrianone, panicutin, denudatine, isotalatizidine and condelphine were present in the root of *D. denudatum.* In contrast, Asif *et al.* (1981) reported only steroids and fat were present in the rhizome of *D. denudatum*. On the other hand, sugar, protein, phenol, starch, iron, zinc, calcium, magnesium and potassium are also present in *D. denudatum* rhizome, which have been reported by Zafar *et al*., 2003. Interestingly, we are the first to report the presence of flavonoids, tannins and terpenoids in the various solvent extracts of the root of *D. denudatum* which were not yet been documented by any researcher.

The developed TLC plate of petroleum ether extract displayed a coloured chromatogram with the universal solvent system, hexane: ethyl acetate; 7: 3, which made the detection easier than other three. Six separate bands were observed on the developed TLC plate of petroleum ether extract. The R_f values of petroleum ether extract bands were 0.44, 0.47, 0.48, 0.53, 0.55 and 0.67 under 254 nm UV irradiation. Whereas, 346 nm UV irradiation showed different bands on TLC were 0.29, 0.44, 0.48, 0.53, 0.54, 0.55 and 0.68. Methanol-H_2SO_4 reagent sprayed TLC plate showed the pink colour bands it indicates the presence of steroids (Figure 1).

In vitro antioxidant activities of DPPH radicals, hydroxyl radicals and superoxide anion radical scavenging activities

of EE, PEE, EAE and CE of *D. denudatum* were shown in Table 2. The scavenging effect of *D. denudatum* extracts on the DPPH radical followed the order: EE< CE< EAE< PEE and was found to be (IC_{50} values) 92.47, 97.66, 104.81 and 119.02 µg/mL, respectively. It was observed that ethanolic extract of *D. denudatum* had higher than that of the other solvent extracts. The IC_{50} value of ascorbic acid (standard) was 3.82 µg/mL. The scavenging effect of *D. denudatum* extracts on the SOS radical followed the order: EE< EAE< CE< PEE and was found to be (IC_{50} values) 314.11, 349.77, 368.05 and 372.49 µg/mL, respectively. However, the ethanolic extract had higher scavenging activity than other solvent extracts. The IC_{50} value of ascorbic acid (standard) was 97.49 µg/mL. The free radical scavenging effect of *D. denudatum* extracts on the hydroxyl radical followed the order: EAE< EE< CE< PEE and was found to be (IC_{50} values) 196.84, 205.44, 219.36 and 225.90 µg/mL, respectively. Interestingly, ethyl acetate extract had higher hydroxyl radical scavenging activity than the other solvent extracts. The IC_{50} value of ascorbic acid (standard) was 90.72 µg/mL. These results revealed that, the ethanolic extract of *D. denudatum* was free radical (DPPH, and SOS) inhibitors acting as primary antioxidants whereas ethyl acetate extract had hydroxyl radical scavenger as a primary antioxidant than other three extracts.

The total phenolic contents in the examined *D. denudatum* root extracts ranged from 58.03 to 47.94 mg GAE/g (Table 3). The highest concentration of phenols was measured in ethanol (58.03 mg GAE/g), chloroform (52.62 mg GAE/g) and ethyl acetate (47.94 mg GAE/g) extracts but absent in petroleum ether extract. The concentration of flavonoids in root extracts from *D. denudatun* ranged from 20.26 to 11.04 mg QE/g. The concentration of flavonoids in methanol extract was 20.26 mg QE/g followed by petroleum ether (17.81 mg QE/g), ethyl acetate (15.19 mg QE/g) and chloroform (11.04 mg QE/g) extracts. The lowest flavonoid concentration was measured in chloroform extract. The total phenolic and flavonoid contents in root extracts of the species *D. denudatum* depends on the type of extract, i.e. the polarity of solvent used in extraction. Phenols and flavonoids are widely found in food products derived from plant sources, and they have possessed significant antioxidant activities (Van Acker *et al.*, 1996). Many studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases (Hertog *et al.*, 1993). Phenol and flavonoid contents of this plant may lead to its good scavenging activity. Further investigation of individual compounds, their *in vivo* antioxidant activities and different antioxidant mechanisms is needed.

Table 1: Preliminary phytochemical constituents' analysis of different extracts of the whole plant of *D. denudatum*

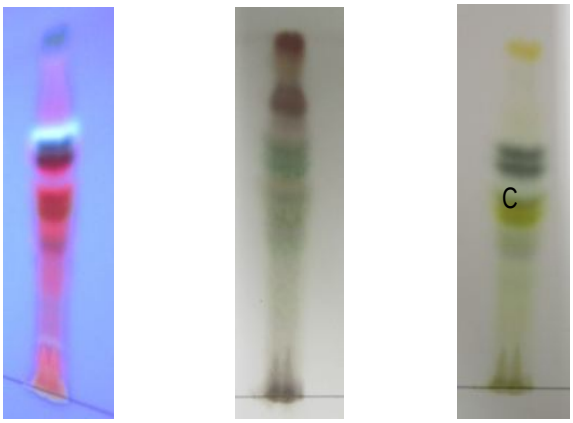
Constituent	Test	EE	PEE	EAE	CE
Alkaloids	Mayer's test	+	-	-	-
	Wagner's test	+	-	-	-
	Hager's test	+	-	-	+
	Dragendorff's test	+	-	-	+
Carbohydrates	Molish's test	+	-	-	-
	Fehling's test	+	-	-	-
	Bradford's test	+	-	-	-
	Benedict's test	+	-	-	-
Glycosides	Borntrager's test	-	+	-	+
Saponins	Foam Test	-	-	-	-
Proteins & Amino Acids	Biuret test	+	-	-	-
	Ninhydrin test	+	-	-	-
Phytosterols & Triterpenoids	Libermann-Burchard's test	+	+	-	+
	Salkowski's test	+	+	-	+
Phenol & Tannins	Ferric Chloride test	+	-	+	+
	Gelatin test	+	-	+	+
	Lead acetate test	+	-	+	+
Flavonoids	Alkaline reagent test	+	+	+	+

Note: + indicates the presence of compound, - indicates the absence of compound. EE- Ethanolic extract, PEE- Petroleum ether extract, EAE- Ethyl acetate extract and CE- Chloroform extract.

Table 2: Comparison of *in vitro* antioxidant status in different solvent crude extracts of *D. denudatum*

Antioxidant Activity	Extracts/Standard (IC_{50} Concentration µg/mL)				
	EE	PEE	EAE	CE	AA
DPPH	92.47	119.02	104.81	97.66	3.82
SOS	314.11	372.49	349.77	368.05	97.49
Hydroxyl Radical Scavenging	205.44	225.90	196.84	219.36	90.72

Note: EE-Ethanolic extract; PEE-Petroleum ether extract; EAE-Ethyl acetate extract; CE-Chloroform extract; AA-Ascorbic acid.



A- Petroleum ether extract of *D. denudatum* visible at 254 nm
B- Petroleum ether extract of *D. denudatum* visible at 346 nm
C- Petroleum ether extract of *D. denudatum* sprarying with methanol H_2SO_4 reagent.

Figure 1: TLC chromatogram of the investigated crude petroleum ether extract of *D. denudatum* rhizome

Table 3: Estimation of total phenols and total flavonoids in different solvent crude extracts of *D. denudatum*

Extracts/ Standard	Total Phenols (mg of GAE/g)	Total Flavonoids (mg of QE/g)
Ethanolic extract	58.03	20.26
Petroleum ether extract	-	17.81
Ethyl acetate extract	47.94	15.19
Chloroform extract	52.62	11.04

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