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Research Article

Influence of Solvent Polarity on Phytochemicals, Antioxidants, and Antimicrobial Properties of *Delphinium denudatum*: A Medicinal Herb from Sainj Valley, Himachal Pradesh, India

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Abstract
Plants with medicinal properties play an important role in pharmaceutical industries for their disease prevention and treatment applications. *Delphinium denudatum*, commonly known as *jadwar*, is an important medicinal plant of the Himalayan region. Therefore, in the present study, the effect of solvent polarity (using seven different solvents i.e., methanol, ethanol, acetone, chloroform, ethyl acetate, hexane, and water separately) was estimated for its secondary metabolites production, antioxidants, and antimicrobial activities. Among the seven different extracting solvents used, the methanol extract of leaf rendered the highest phenolic content (80.52 mg GAE/g (dry weight (dw)). Acetone extracts for the shoot were found to be most efficient with the extraction of the highest flavonoid content (57.53 mg QE/g (dw) while the methanol extract of root rendered the highest tannin content 18.78 mg TAE/g (dw). Likewise, the methanol extract of the leaf showed the highest flavonol content 34.76 mg QE/g (dw). For antioxidant activity, the IC_{50} value for ABTS activity ranged from 35.15 to 103.08 µg/mL, and for DPPH activity it was 75.23 to 256.21 µg/mL. Further, all the plant parts i.e., leaf, shoot, and root, showed antimicrobial activity against *Bacillus subtilis, Escherichia coli,* and *Serratia marcescens* having MIC between 400 to 900 µg/mL. Among all the tested plant parts, polar solvent leaf extracts had higher antioxidant activity. Furthermore, phenols, flavonols, tannins, and ABTS activity from leaf, shoot, and root have shown a positive relation with solvent polarity. In all three plant parts; phenols, flavonols, and tannin positively correlate with antibacterial activity. The present study further revealed that the secondary metabolites in the leaf, shoot, and root extracts of *D. denudatum* are an excellent source of antioxidant and antimicrobial activities, thus validating the species' therapeutic potential.

Keywords: *Delphinium denudatum,* medicinal herb, traditional medicine, antioxidants, antimicrobials

1. INTRODUCTION

Since natural products are more affordable and have fewer negative effects, their use is becoming more and more popular. Since plant extracts are said to be a source of several phytochemicals, natural antioxidants, and antimicrobials, they are being investigated for potential novel drugs [1]. Moreover, plants with medicinal properties play an increasingly important role in pharmaceutical industries for their functions in disease prevention and treatment by having potential antimicrobial activity [2]. A large group of compounds produced by plants referred to as phytochemicals possessing high antioxidant properties have been seen to help tackle numerous diseases. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including heart and cancer diseases [3].

Phytochemical antioxidants are very efficient scavengers of free radicals. Antioxidants are those substances that possess free radicals chain reaction-breaking properties. Plant products can be attributed to the biological activities of their phytochemicals and antioxidant constituents such as phenolic, proanthocyanidin, vitamins, carotenoid, flavonoid, and saponin compounds [4]. Medicinal plants, especially the endemic and edible plants of a region, due to their ability to produce natural compounds with antioxidant capacity and antimicrobial properties and due to their health benefits are particularly important for the development of new drugs. In this perspective, plant-based antimicrobials (derived from medicinal plants, in particular) are increasingly receiving attention for harnessing their potential in the production of antimicrobial substances, as safer sources of antibiotics. Crude extracts and essential oils of medicinal plants possess bioactive compounds, often with antimicrobial and antioxidant properties [5]. Antimicrobial

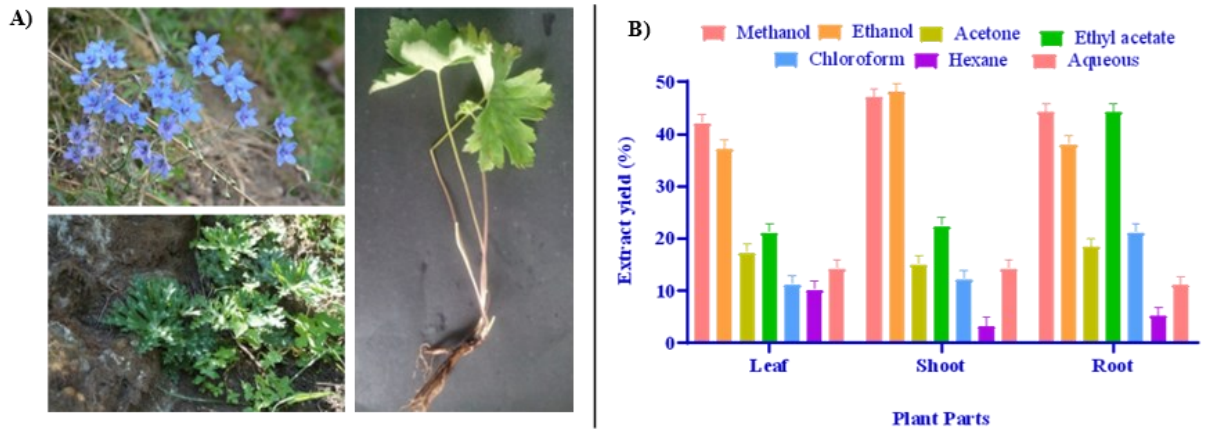

Figure 1. A) Images of *D. denudatum* in the wild and plant images (including all plant parts) in the lab. B) Extractive yield of *D. denudatum* different plant parts (Leaf, Shoot, and Root).

compounds are used in various areas such as pharmaceuticals, neutraceuticals, textiles, dairy products, cosmetics, and personal care products [6].

Delphinium denudatum Wall (family Ranunculaceae) is an important medicinal plant commonly known as *jadwar*. It provides one of the important drugs used as indigenous medicine in India, especially in Unani medicines [7][8]. It is a perennial branched erect herb found in Northwest Himalayas. Roots of *D. denudatum* are used for the treatment of toothache, rheumatism, syphilis, snakebite, and aconite poisoning. It is also used as an alternative, tonic, and in the treatment of epilepsy in the Unani healthcare system [9]. Images of *D. denudatum* from the wild and in the laboratory are shown in Figure 1A. Ethanol and aqueous extracts are reported to have morphine addiction activities [10][11]. The ethanolic extract is also reported to have a protective effect in rat models of Parkinson's disease [12]. The roots are reported to contain diterpene alkaloids of cuisine and veatchite types [13]. Furthermore, before the extraction of antioxidant and antibacterial compound(s), the influence of solvent polarity and optimization of plant extractive values is particularly critical. Researchers have also focused on choosing appropriate extraction solvents and extraction techniques for evaluating bioactive substances, such as antimicrobials, from various medicinal plants and plant parts [6]. Despite the high demand and widespread use of *D. denudatum*, there aren't many papers on the plant. For this reason, the current work focuses on a comprehensive analysis of how solvent polarity impacts phytochemical, antioxidant, and antibacterial activities. We have concentrated on solvent polarity from polar to non-polar in this work because it is an important first step in the extraction process of secondary metabolites. Its basic phytochemical and antibacterial activities make it an important medicinal plant. This is important since the species grows throughout a wide range of altitude and habitat types. Given the current context, the study aims to examine how different solvent systems affect the phytochemicals, antioxidants, and antibacterial qualities of *D. denudatum* plant parts.

2. MATERIALS AND METHODS

2.1. Study Site and Sample Collection

The fresh and healthy root, stem, and leaves of *D. denudatum* were collected from Sainj Valley of Kullu District in Himachal Pradesh (31º45.881' N to 31º76.897' N latitudes and 77º19.031' E to 77º33.747' E longitudes) during May and June 2021; samples were brought to the laboratory, air dried, converted into a fine powder, and stored at 4-8 °C for the further analysis.

2.2. Methods

2.2.1. Extraction

Variation in extraction methods usually depends on the length of the extraction period, the solvent used, and the solvent-to-sample ratio. The basic principle is to grind the plant material 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 plant tissue in various solvents. Root, leaf, and stem were extracted (separately) in seven solvents (methanol, ethanol, acetone, chloroform, ethyl acetate (E. acetate), hexane, and water separately) taking in a ratio of 1:5 (dry powder: solvent). The mouth of the conical flask was sealed with parafilm. Samples were macerated in a rotary shaker (Remi) at 160 rpm for 48 h.

2.2.2. Phytochemical Properties of D. denudatum Extracts

2.2.2.1. Total Phenols

The total phenolic content of the extract was determined by the Folin-Ciocalteu method [14]. Briefly, 200 µL of crude extract (1 mg/mL) was made up to 3 mL with distilled water, mixed thoroughly with 0.5 mL of Folin-Ciocalteu reagent for 3 min, followed by the addition of 2 mL of 20 % (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per gram of dry weight (mg/g (dw)).

2.2.2.2. Total Tannins

The tannin content of the extract was determined by the Folin-Denis method [15]. As much as 50 µL of crude extract was mixed properly with 0.5 mL of Folin Denis reagent, followed by the addition of 7% sodium carbonate. Then, 3 mL of distilled water was added to it and the mixture was allowed to stand for a further 20 min and absorbance was measured at 700 nm. The tannin content was calculated from the calibration curve, and the results were expressed as mg of tannic acid equivalent per g dry weight.

2.2.2.3. Total Flavonoids

The aluminum chloride colorimetric method was

Table 1. Qualitative phytochemical analysis of *D. denudatum* different plant parts extracts.

Plant parts	Solvent	Phenolics	Flavonoids	Terpenoids	Alkaloids	Glycosides	Saponins	Protein	Carbohydrates
	Methanol	++++	++++	++++	++++	++++	++++	++	++
	Ethanol	++	++	++	++	++	-	++	++
	E. acetate	++	++	+++	++	++	+++	++	++
Leaf	Acetone	+++	++	-	+	+	-	++	++
	Chloroform	+	+	++	-	+	++	-	-
	Hexane	+	+	++	-	+	-	++	++
	Aqueous	++	++	++	+	+	+++	+	+
	Methanol	++++	++++	++++	++++	++++	++++	++	++
	Ethanol	+	+	++	+	++	-	++	++
	E. acetate	++	++	+++	++	++	+++	++	++
Shoot	Acetone	+++	++	-	-	+	-	++	++
	Chloroform	+	+	++	-	+	++	-	-
	Hexane	+	+	++	-	+	-	++	++
	Aqueous	++	++	++	+++	+	+++	+	+
	Methanol	++++	++++	++++	++++	++++	++++	++	++
	Ethanol	+	++	+++	-	++	-	++	++
	E. acetate	++	++	+++	++	++	+++	++	++
Root	Acetone	+++	++	-	-	+	-	++	++
	Chloroform	+	+	++	-	+	++	-	-
	Hexane	+	+	++	-	+	++	++	++
	Aqueous	++	++	++	++	+	+++	+	+

Note: - = negative, + = positive, number of + denotes colour intensity. + + = light; + + + =medium; and + + + + =dark colour production

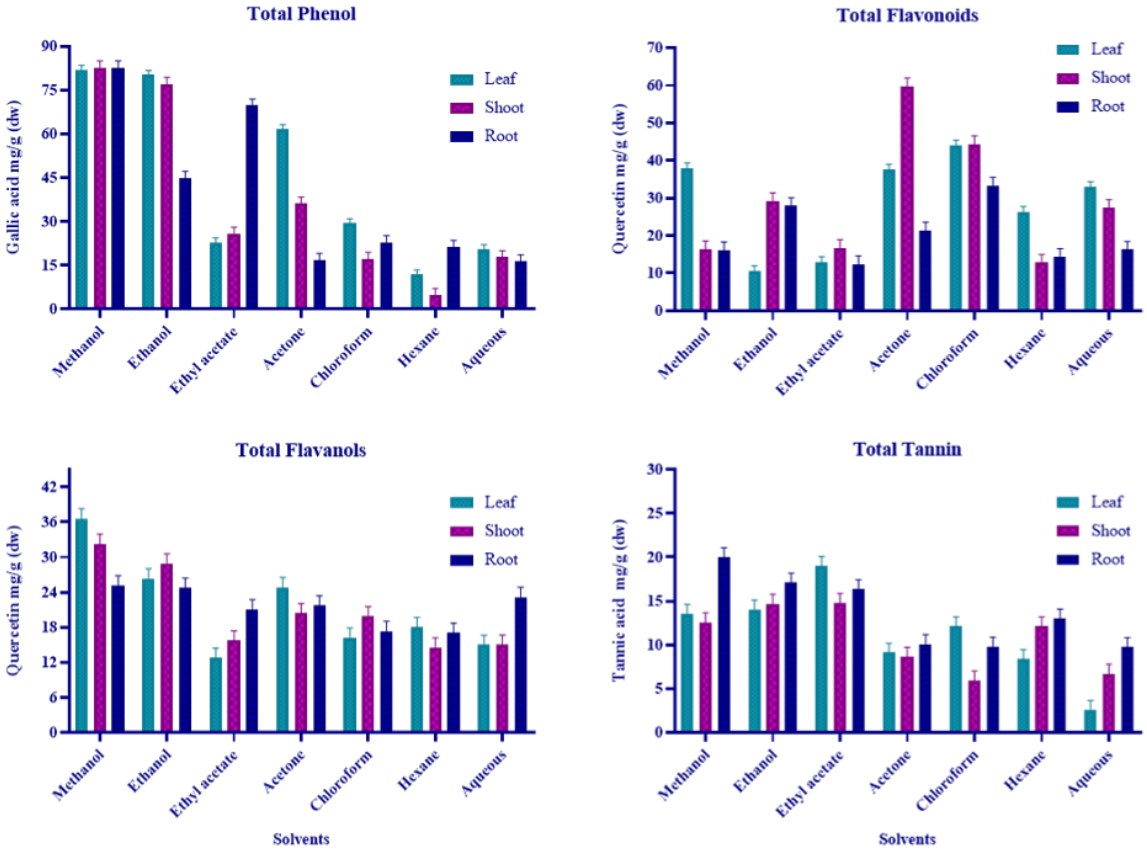

Figure 2. Phytochemical analysis of *D. denudatum* different plant parts extracts.

used for the determination of the total flavonoid content of the sample [16]. As much as 0.5 mL of the extract (5 g/L) was mixed with 1.5 mL of methanol and then, 0.1 mL of 10% aluminum chloride was added, followed by 0.1 mL of potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 min. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g dry extract). Quercetin was used as positive control and the standard curve was prepared by quercetin in different concentrations.

2.2.2.4. Total Flavonols

The flavonol content of the samples was determined since flavonols represent the main fraction of compounds responsible for the antioxidant activity. The content was estimated according to Tapan [17]. As much as 2.0 mL of the sample in ethanol was mixed with 2.0 mL $AlCl_3$ (2%) in ethanol (96%) and 3.0 mL sodium acetate solution (50 g/L). The absorbance at 440 nm was recorded after 2.5 h incubation at 20 °C. The results were expressed as milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g dry extract). Quercetin was used as positive control and the standard curve was prepared by quercetin in different concentrations.

2.2.3. Antioxidant Activity

2.2.3.1. DPPH Assay

The free radicals-scavenging potential of crude extracts was determined using a DPPH assay [18]. A 0.50 µL aliquot of the extract solution was mixed thoroughly with 2.5 mL of 0.3 mM DPPH prepared in methanol for 1 min and then allowed to stand at room temperature for 20 min. Absorbance was measured at 517 nm. Quantifying radical activity was determined based on a standard curve of ascorbic acid prepared in methanol.

2.2.3.2. ABTS Assay

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS) assay was carried out according to the method of Amarowicz et al.

[19]. $ABTS^{\cdot+}$ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulphate (1:1), stored in the dark at room temperature for 12–16 h before use. $ABTS^{\cdot+}$ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µL of plant extract to 3.995 mL of diluted $ABTS^{+}$ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay.

2.2.4. Antimicrobial Activity

2.2.4.1. Qualitative Test (Plate based Bioassay)

The bacterial cultures, Gram-positive i.e., *Bacillus subtilis*, and Gram-negative namely *Escherichia coli* and *Serratia marcescence* were used as test organisms in this investigation. The media used for the antibacterial test was tryptone yeast extract Agar. The antibacterial activity was carried out by employing 24 h of cultures [6]. The activity of all the extracts was screened for antibacterial activity and was tested separately using the disk diffusion method. About 30 mL of the agar medium with respective strains of bacteria was transferred aseptically into each sterilized Petri plate. The extracts were placed in a 6 mm diameter. Antibacterial assay plates were incubated at 37 °C for 24-48 h and the diameter of the zone of inhibition was measured.

2.2.4.2. Quantitative Test (Minimum Inhibitory Concentration)

Bacterial culture suspensions were prepared in TYE broth. For determination of MIC, 500 µL standard compound solution of different concentrations ranging from 10 to 50 µg/mL was diluted using 500 µL test organism and 4 mL TYE broth in the sterile test tube and then incubated at 27 °C for 24-48 h. Control was prepared in two sets, one containing broth medium and test organism while the other containing broth medium and extract [6]. After 24 h, the MIC values were recorded based on the lowest concentration showing an absence of growth in the tubes. The test was further confirmed by plating on TYE agar.

2.2.5. Statistical Analysis

Estimating phytochemical compounds (total phenolics, tannins, flavonols, and flavonoids), and antimicrobial and antioxidant activities by ABTS and DPPH were conducted in triplicates. The data expressed as the means ± standard errors (SE) from experiments performed in triplicate. Statistical significance and mean between groups were tested using Student's T-test and two-way ANOVA. The p -value <0.05 was considered significant. Pearson's correlation was calculated among different parameters used in the present study using R software (R-3.4.1).

3. RESULTS AND DISCUSSIONS

3.1. Extraction Yield

The extraction yield varied with different solvents, and the highest extraction yield was obtained in polar protic solvents such as methanol (45.21± 0.75% in shoot, 42.35± 0.84% in root, and 40.23 ± 0.89% in leaf) and ethanol (46.25± 0.86% in shoot, 36.12± 0.71% in root, and 35.26± 0.73% in leaf) than polar aprotic solvents such as acetone (16.48± 0.26% in root, 15.32± 0.29% in leaf, and 13.15± 0.32% in leaf) and ethyl acetate (42.39± 0.69% in roots, 20.45± 0.69% in shoot, and 19.23± 0.49% in leaf). The extraction yield showed a clear linkage between the polarity of the solvent and yield. The polar protic solvent (methanol and

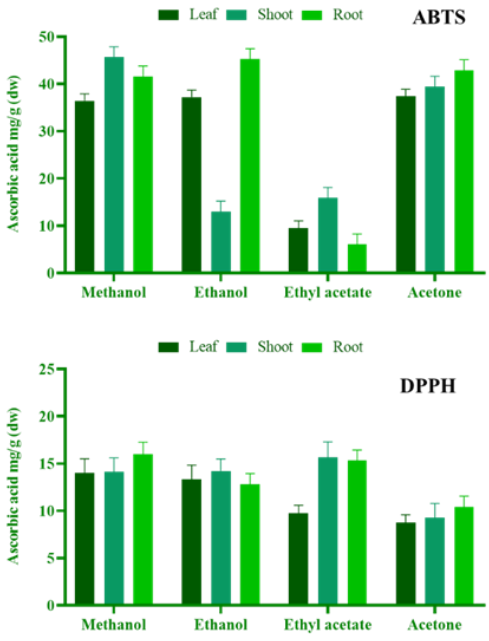

Figure 3. Effect of solvent polarity in antioxidants potential of *D. denudatum* different plant parts extracts.

Table 2. Antioxidant activity of *D. denudatum* different plant parts extracts.

Solvent	Plant Parts	IC_{50} (µg/mL) ABTS	IC_{50} (µg/mL) DPPH
Methanol	Leaf	50.63±0.55	70.23±0.95
	Shoot	35.15±0.95	95.19±0.75
	Root	35.26±1.09	105.32±1.56
Ethanol	Leaf	35.26±0.75	75.23±0.92
	Shoot	60.24±0.72	90.10±0.79
	Root	49.32±1.18	106.32±1.06
Ethyl acetate	Leaf	95.32±0.65	95.32±0.29
	Shoot	63.12±0.98	165.23±0.75
	Root	54.23±1.06	115.23±1.32
Acetone	Leaf	86.08±2.15	135.26±0.67
	Shoot	103.08±1.26	156.32±0.49
	Root	125.25±1.42	256.21±2.35

ethanol) extracted a significantly greater yield than the polar aprotic (acetone, aqueous, and ethyl acetate) and non-polar (chloroform and hexane) solvents. The highest yield in methanol was due to the dissolving efficacy of the organic compound with a protonatable functional group and low-molecular-weight organic compound groups [6] [20]. Results on extract yield are shown in Figure 1B.

3.2. Phytochemical Analysis

Phytochemical analysis showed the presence and absence of certain chemical constituents in different extracts such as methanol, ethanol, ethyl acetate, acetone, hexane, chloroform, and water. The total phenolic, flavonoids, flavonol, and tannin content of sample extracts obtained from different plant parts of *Delphinium denudatum* varied with the solvent used for the extraction (Table 1). The concentration of total phenolic in the examined extract ranged from 10.45 to 80.52 mg GAE/g (dw). Among the seven different extracting solvents used, the methanol extract of the leaf rendered the highest phenolic content of 80.52 mg GAE/g (dw) followed by the ethanol extract of the leaf at 78.73 mg/GAE/g (dw), ethanol extract of shoot 74.84 mg GAE/g (dw), ethyl acetate of root 67.45 mg GAE/g (dw) while aqueous and hexane extracts showed the lowest phenolic contents than those of other solvents (Figure 2).

Plants are rich sources of active metabolites like tannins, alkaloids, flavonoids, phenols, steroids, etc. which are responsible for their therapeutic activities [4]. Plants are a potent source of phytochemical constituents that are responsible for their pharmacological activities. Many phytochemical compounds, such as phenolic, flavonoids, tannin, and alkaloids have been isolated from various plant species [1]-[3]. Flavonoids, tannins, and phenolic substances are constituents of plants with potential antioxidant activity, mainly because they act as free radical scavengers [21]. The total flavonoid content values examined ranged from 8.99 to 57.53 mg QE/g (dw). Amongst all the solvents tried acetone extract for the shoot was found to be most efficient with the extraction of the highest flavonoid content (57.53 mg QE/g (dw)), followed by chloroform of leaf (42.40 mg/QE/g (dw)) and chloroform of shoot (42.14 mg/QE/g (dw)) and ethyl acetate extract showed the lowest flavonoid content than those of other solvents (Table 1 and Figure 2).

The concentration of tannin in the examined extract ranged from 1.37 to 18.78 mg TAE/g (dw). Among the seven different extracting solvents used the methanol extract of root rendered the highest tannin content of 18.78 mg TAE/g (dw) followed by ethyl acetate extract of leaf at 17.79 mg/TAE/g (dw), ethanol extract of root 15.87 mg TAE/g (dw), ethyl acetate of root 15.13 mg TAE/g (dw), and aqueous extract showed the lowest tannin contents than those of other solvents (Figure 2). Figure 2 showed that the total flavonol concentration of different extracts ranged from 10.83 to 34.76 mg QE/g (dw). The methanol extract of the leaf showed the highest flavonol content 34.76 mg QE/g (dw), followed by the methanol extract of the shoot

(30.40 mg QE/g (dw)), and ethyl acetate extract showed the lowest flavonol contents as compared to other solvents. According to Mohanpriya and Siva [22], the phytochemical screening of different solvent extracts (like flavonoids, tannin, phenolic, etc.) of the root of *D. denudatum* revealed the presence of medically bioactive constituents and tannin is absent in the *D. denudatum* ethanolic extract. However, the present study showed that tannin is present in the ethanolic extract of *D. denudatum.*

Medicinal plants can be used in fresh or dried form. However, drying is the most common method for the post-harvest preservation of medicinal plants and must be accomplished as soon as possible after harvesting to increase the quality of plants and prevent the expected contamination and losses [23]. Previous study showed the presence of high phenolics and alkaloid content in *Delphinium malabaricum* root extracts whereas the leaf extract exhibited the presence of high flavonoid content [24], but in the present study, *D. denudatum* showed the presence of high phenolic content in leaf extracts. Interestingly, this is the first report on the presence of total phenolics, flavonoids, tannins, and flavonols in the various solvent extracts of the leaf, shoot, and root of *D. denudatum* which have not yet been documented by many researchers.

3.3. Antioxidant Activity

DPPH is a stable free radical at room temperature and accepts an electron, and hydrogen radical to become stable diamagnetic molecules [25]. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants. On the other hand, ABTS is an excellent substrate for peroxidase and is frequently used to study the antioxidant properties of natural compounds [26]. Antioxidants are essential substances that possess the ability to protect the body from damage caused by free radical-induced oxidative stress. Medicinal plants are a rich source of phytochemicals, such as carotenoids, flavonoids, and other phenolic compounds having high free-radical scavenging activity, which helps to reduce the risk of chronic diseases, such as cardiovascular disease, cancers, and neuronal degeneration [27].

In the present study, the plant extracts' shoot and leaf were examined for their radical scavenging properties of DPPH and ABTS. Results showed that among all the tested solvent extracts, methanol, ethanol, ethyl acetate, and acetone showed antioxidant potential. For antioxidant activity, the methanol extract of the shoot showed the highest antioxidant value at 43.44±0.96 mg AAE/g (dw) followed by the ethanolic extract of root at 43.00±0.53 mg QE/g (dw), and ethanolic extract of leaf 35±0.44 mg QE/g (dw). Likewise for DPPH scavenging activity, the highest activity present in ethyl acetate extract of shoot was 15.43±0.032 mg AAE/g (dw) and root 15.25±0.068 mg AAE/g (dw), and methanol extract of leaf 12.48±0.0105 mg AAE/g (dw) (Figure 3). IC_{50} value for ABTS activity is ranged from 35.15 to 103.08 µg/mL and for DPPH activity it is 75.23 to 256.21 µg/mL (Table 2). Comparable outcomes were noted for several therapeutic plants, including *Celastrus peniculatus Larrea tridentate*, *Peltophorum ferrugineum*, and *Ocimum gratissimum* [28][29].

Table 3. Antimicrobial Activity *D. denudatum* different plant parts extracts.

Solvent	Plant Parts	*Bacillus subtilis*		*Escherichia coli*		*Serratia marcescens*	
		Zone of inhibition	MIC (µg/mL)	Zone of inhibition	MIC (µg/mL)	Zone of inhibition	MIC (µg/mL)
Methanol	Root	3.00±0.12	500	3.33±0.04	500	4.67±0.11	400
	Shoot	2.33±0.11	700	1.67±0.10	500	2.61±0.08	900
	Leaf	1.67± 0.04	800	6.67 ± 0.08	500	8.00±0.51	500
Ethanol	Root	2.33±0.10	700	4.13±0.25	600	9.33±0.18	300
	Shoot	7.00 ± 0.35	400	5.33±0.08	500	15.33± 0.11	300
	Leaf	3.00± 0.07	600	6.67 ± 0.08	500	6.33 ± 0.36	300
Chloroform	Root	2.33±0.11	900	4.67±0.15	700	2.00±0.07	900
	Shoot	2.00±0.07	900	ND	ND	ND	900
	Leaf	2.00±0.07	900	4.33±0.15	600	3.67±0.18	800

MIC= minimum inhibitory concentration; Zone of inhibition = mm; ND= not detected

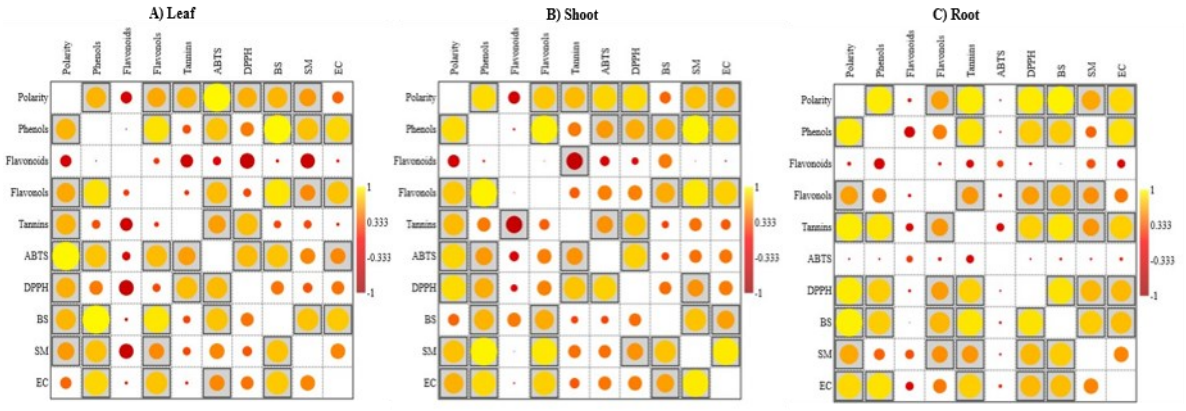

Figure 4. Pearson's Correlation among solvent polarity, phytochemical, antioxidants, and antimicrobial activity of different plant parts of *Delphinium denudatum.* Correlation is significant at 0.05 level, grey highlighted box=p<0.05, simple box=p>0.05. ABTS= 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), DPPH= 2,2-Diphenyl-1-picrylhydrazyl, BS= *Bacillus subtilis*, SM= *Serratia marcescens* and EC=*Escherichia coli*.

3.4. Antimicrobial Activity

All three plant parts (root, shoot, and leaf) were used for testing the antibacterial activity against both Gram-positive i.e., *B. subtilis,* and Gram-negative bacteria i.e., *E. coli* and *S. marcescens.* Ethyl acetate, chloroform, and aqueous extracts showed selective antibacterial activity. Results in detail are given in our previous study [30]. Moreover, methanolic, ethanolic, and chloroform extracts showed antimicrobial activity against all the tested bacteria. Only chloroform extracts of shoot didn't show activity against Gram-negative bacteria i.e., *E. coli* and *S. marcescens.* Results on the zone of inhibition (mm) are shown in Table 3. Methanol and ethanol extracts are reported for good antibacterial potential in previous reports also from *Taxus wallichiana* [6] and *Curcuma caesia* [3]. Quantitative analysis was also done by calculating the MIC of all the plant extracts having the zone of inhibition. MIC ranged between 500 to 900 µg/mL for *B. subtilis,* 500 to 600µg/mL for *E. coli,* and 400 to 900 µg/mL for *S. marcescens.* MIC in the same range was also reported from leaf, rhizome and *in vitro* propagated callus of *Paeonia emodi* [1].

3.5. Pearson's Correlation

A Pearson's correlation analysis was conducted between various metrics, including solvent polarity, phytochemicals, antioxidant activity, and antibacterial activity of individual plant components. The outcomes are displayed in Figure 4. The phenol concentration and ABTS activity of leaf solvent were positively linked with polarity. Phenolic and flavonoid content positively correlated with antimicrobial activity. Further, solvent polarity, phenol concentration, ABTS, and DPPH activity were all positively connected with solvent polarity. Phenolic and flavonoid content positively correlated with antimicrobial activity. Likewise, phenol, tannin concentration, ABTS activity, and antibacterial activity were all positively connected with solvent polarity in roots. Phenolic and tannin concentrations positively correlated with antimicrobial activity.

It has been found that phenol and flavonoids possessed antibacterial properties against both Gram-positive and Gram-negative bacteria. Phenolic compounds' primary mechanisms of action include damaging bacterial cell membranes, preventing the development of virulence traits, and preventing the formation of bacterial biofilm [31]. Different processes are used by flavonoid compounds to eliminate microorganisms. The presence of the phenolic hydroxyl group in flavonoids has been shown to have a key role in inhibiting the formation of microbial enzymes and simultaneously destroying the cell wall leading to cell death. This is achieved by increasing the protein binding affinity of flavonoids [32]. By promoting the breakdown of the plasma membrane and impeding the growth of cell walls and protein synthesis, flavanols eradicate fungi [33].

4. CONCLUSIONS

This is the first report to assess how different solvents affect the antioxidants, antimicrobial activity, and phytochemical compositions of leaf, shoot, and root extracts of *D. denudatum*. The results of this work indicate that more secondary metabolites with potential for antioxidant and antibacterial activities can be produced by synthesizing secondary metabolites in *D. denudatum* leaf, shoot, and root extracts in polar solvents. The plant extracts of the leaves, shoots, and roots of *D. denudatum* are rich in antioxidants and antibacterial compounds that can be applied to pharmaceuticals, healthcare goods, medicinal applications, and nutritional needs (such as food additives and preservatives). Future research should be done to find out whether they have antidiabetic, antiproliferative, and anticancer properties. Antioxidants and antimicrobials can be extracted from the leaves and added to culinary and medical items. This will support the preservation of it as a non-destructive harvesting technique.

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Author Contributions

K. K. and P. A. data curation, analysis, and paper writing. M. L. plant sample collection. A. P. and S. S. S. conceptualization of data, and paper editing. K. K. and P. A. contribute equally.

Conflicts of Interest

The authors declare no conflict of interest.

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