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RESEARCH ARTICLE

Antioxidative potential of *Angelica glauca* Edgew. and *Dioscorea deltoidea* Wall.: high value, endangered medicinal herbs of north west Himalaya

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Abstract

Reactive oxygen species (ROS) generated during aerobic metabolism cause oxidative damage leading to several disorders, ageing or cell death. Plants synthesize an array of phytochemicals having strong antioxidative properties that can delay or prevent oxidative damage. Here, we monitored the antioxidative properties of different plant parts of two high-valued, endangered medicinal plant species: *Angelica glauca* Edgew. (Apiaceae) and *Dioscorea deltoidea* Wall. (Dioscoreaceae) from Kinnaur, Himachal Pradesh, India, using DPPH (1, 1-diphenyl-2-picrylhydrazyl) and pyrogallol autoxidation assays. The underground parts (roots/rhizomes) of both the species exhibited substantially greater antioxidative activity as compared to the other plant parts. Further, of the two tested extraction solvents, methanolic extracts exhibited higher activity as compared to the aqueous extracts across all considered plant parts in both the species implying the variability in phytochemical composition of the extracts. We compared the observations with those reported earlier and discussed the potential of *A. glauca* and *D. deltoidea* as a source of natural antioxidants.

Keywords: Antioxidants, *Angelica glauca, Dioscorea deltoidea,* DPPH, Medicinal plants, Pyrogallol.

Introduction

The generation of reactive oxygen species (ROS) or oxygen free radicals (FR) comprises an inevitable component of aerobic metabolism. Being highly reactive, ROS combine with diverse biological molecules adversely affecting them. The long-term exposure to ROS can cause potential oxidative damage that is considered to be the major cause of ageing and/or cell death (Nagel *et al*. 2019; Huang and Li 2020). Besides ageing, they cause several disorders including auto-immune diseases, cardiovascular malfunction, cancers, cataracts, rheumatism, and many other (Valko *et al.* 2007). The cell organelles such as chloroplasts, mitochondria and peroxisomes play a major role in the production of ROS. They also generate due to the activities of various oxidases e.g., NADPH oxidase, polyphenol oxidases, glycolate oxidase etc. (Sandalio and Romero-Puertas 2015; Dietz *et al.* 2016; Huang *et al*. 2016). The levels of ROS known to increase significantly under various stress conditions which further enhance the cell damage and/or aging process (Sharma *et al.* 2012; Mansoor *et al.* 2022). Nevertheless, in a range of certain lower concentrations, ROS act as signaling molecules, regulating various cellular activities (Huang and Li 2020). The levels of ROS are strongly regulated by various antioxidative enzymes including catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and by non-enzymatic antioxidative compounds. The latter include ascorbic acid, tocopherol, flavonoids, phenolic acids, polyphenols, glutathione and various other secondary metabolites (phenolic compounds, terpenoids and alkaloids) having high antioxidative potential (Das and Roychoudhury 2014). Most of the medicinal plants generally contain high levels of such secondary metabolites and are therefore expected to possess high antioxidative potential.

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In recent years, the natural antioxidants, present in the form of various classes of phytochemicals in medicinal plants, have received much attention due to their strong free radical scavenging potential proven through various in-vivo and in-vitro experiments (Kim *et al*. 2003; Škrovánková *et al*. 2012; Shebis *et al*. 2013; Xu *et al*. 2017; Bhatt *et al*. 2018; Singh and Patra 2018; Naseem *et al*. 2024). Therefore, we evaluated the antioxidative potential of different plant parts of *Angelica glauca* and *Dioscorea deltoidea*, two highly valued medicinal plants from Kinnaur (Himachal Pradesh) (Fig. 1A and B)

Angelica glauca Edgew. (Apiaceae), commonly known as Chora is a perennial herb, endemic to Himalayan region. It is a source of several bioactive molecules and essential oils including alkaloids, flavonoids, phenylpropanoids, phthalides, monoterpenes, coumarins, several saponins and sterols (Kumar *et al*. 2022). The plant is used to cure many diseases/disorders and is recommended as tonic. It possesses cardio-active, carminative, diaphoretic, anti-oxidant, anti-microbial, anti-mutagenic, anti-inflammatory and immunomodulatory activities. Besides, it improves lactation in cattle (Butola and Badola 2004; Purohit *et al*. 2015; Sabeel *et al*. 2023). The species is also in high demand for cosmetics, perfumes, spice and condiments (flavoring dishes, local alcoholic beverages and bitters) (Sen and Thakur 2021; Kumar *et al*. 2022). *Dioscorea deltoidea* Wall. (Dioscoreaceae) or Dioscorea or Singli-mingli (local name) is a perennial herbaceous climber having tuberous roots. It is also known as wild yam and is rich in steroidal sapogenins **(**4.8-8%), mainly diosgenin that is a precursor for the synthesis of many steroid molecules including cortisones, corticosteroids, progesterone and oral contraceptives (Narula *et al*. 2007; Sautour *et al*. 2007)**.** Steroidal drugs find wide application in modern medicine for curing many serious ailments such as, rheumatoid arthritis, allergic conditions, ulcers, muscular dystrophy and cancer. They are also used for the treatment of digestive disorders, diarrhea, irritability, abdominal pain, anemia and possess antimicrobial, antioxidative and hypoglycemic activities (Chandra *et al*. 2012; Obidiegwu *et al*. 2020; Wang *et al*. 2023).

The conservation assessment and management prioritization (CAMP) workshop, 1998 (Molur and Walker 1998) categorized both the species as critically endangered. However, the other CAMP workshops held later (Ved *et al*. 2003; CAMP report 2010) categorized them as an endangered species due to habitat loss, overexploitation and trade. *D. deltoidea* has also been included among the list of 29 species, banned from export unless or until accompanied by a certificate of origin from cultivation. The overexploitation due to increased demand/trade directly or indirectly confirms their high medicinal importance.

The levels of various specific secondary metabolites possessed or synthesized by plants generally determine their medicinal quality and effectiveness. Under certain stress conditions, as a part of adaptive strategies, the levels of secondary metabolites are known to increase considerably (Muthusamy and Lee 2024). In the current study, both the species (*A. glauca* and *D. deltoidea*) included were collected from their natural habitats in District Kinnaur (Himachal Pradesh) where the species generally experience various abiotic stresses including drought, temperature fluctuations and high UV radiations. They are thus expected to exhibit comparatively higher antioxidative properties. As such, the population specific differences in the antioxidative potential of the medicinal plants need to be understood. Therefore, we analyzed the antioxidative properties of aqueous and methanolic extracts of different plant parts (leaves, roots and seeds) of *A. glauca* and *D. deltoidea* from Kinnaur (Himachal Pradesh, India). The results are expected to have implication for *A. glauca* and *D. deltoidae* as a source of potent natural antioxidant.

Material and methods

Plant material

Different plants parts (seeds, leaves and roots/rhizomes) of *A. glauca* and *D. deltoidea* were collected from Nichar (2110 m asl), District Kinnaur, Himachal Pradesh, India in the months of September-October, 2016 and subsequent years. Following collection, the plant parts were completely dried under shade for 10-15 days and thereafter, grinded into fine powder, kept in air tight container at 4 °C in refrigerator till further use.

Preparation of extract

1 g powder of respective plant parts (leaves, seeds and roots/rhizomes) were suspended in 100 ml (final volume) of distilled water and methanol, respectively in an air-tight glass container for 24 h at 37 °C. Thereafter, the suspension was filtered and the filtrate thus obtained was kept in an oven (35 ± 5 °C) for vaporization to get the residue. After complete evaporation, the residue obtained was dissolved in respective solvents (water and methanol). Dissolved extracts were stored at 4 °C till further analysis. Desired concentrations (25 to 400 µg residue/ml) were obtained through appropriate dilution.

DPPH radical scavenging activity

The assay is based on the measurement of the scavenging ability of the antioxidants towards the stable radical DPPH (1, 1-diphenyl-2-picrylhydrazyl). A reaction mixture containing 1 ml of extract and 1 ml of 0.1 mM DPPH (prepared in methanol) was incubated for 30 min. at 25 °C under complete darkness. The activity was monitored through change in absorbance due to decolorization of reaction mixture with a spectrophotometer at 517 nm against a water/ methanol blank. The DPPH radical scavenging activity was calculated using the following equation:

DPPH radical scavenging (%) = $[A_C\text{-}A_S/A_C] \times 100$

Where, A_C= absorbance of control and A_S = absorbance of sample

Pyrogallol autoxidation assay

Pyrogallol auto-oxidizes rapidly forming superoxide radical and lemon-yellow colored product. The latter can be measured spectrophotometrically. Thus, absorbance of reaction mixture containing 1 ml of Tris-hydroxymethyl aminomethane (pH 8.0, added with 1 mM EDTA) and 25 µl of 48 mM pyrogallol (prepared in 1 mM HCl) with or without plant extract was measured at 420 nm, after every 30 seconds up to 5 min at room temperature. The change in absorbance (ΔA) was measured (the absorbance at 5 min – the absorbance at 1 min) and the $\bullet O^{2-}$ scavenging ability of plant extract (inhibition of pyrogallol autoxidation) was determined using the following equation (Marklund and Marklund, 1974):

$$\text{Inhibition of pyrogallol autoxidation}(\%) = \frac{\Delta A_{420C} - \Delta A_{420S}}{\Delta A_{420C}} \times 100$$

Where,

ΔA_{420C} = change in absorbance of the mixture without plant extract and

ΔA_{420S} = absorbance change in the presence of plant extract

Results

DPPH radical scavenging activity

The assay is based on measurement of the scavenging ability of antioxidants towards the stable free radical, DPPH·. Plant extracts possessing the antioxidative activity reduce the stated radical to its corresponding hydrazine upon reaction with the hydrogen donors present in the antioxidative principles. The radical scavenging activity of both water and methanol extracts of different plant parts of *A. glauca* increased with increasing concentrations. At 25 µg/ml aqueous extract, the radical scavenging activity was found to be 28, 21 and 25% in root, leaf and seed extract of *A. glauca*, respectively. With the increase in concentration, the activity increased correspondingly; thus, at 400 µg/ml concentration, 53, 38 and 46% radical scavenging activity was evident (Fig. 2A). In case of methanolic extract of *A. glauca*, the values for radical scavenging activity at 25 and 400 µg /ml concentrations were 37 and 69 (in root), 32 and 54 (in leaf) and 30 and 53% (in seed), respectively (Fig. 2B). The roots exhibited marginally greater antioxidative activity as compared to other parts.

The DPPH reducing potential of different parts of *D. deltoidea* also exhibited concentration dependent effect with both aqueous and methanolic extracts (Fig 3). In a concentration range between 25 to 400 µg/ml aqueous extract, the radical scavenging activity was found to be 14-64, 11-56, and 9-46 % in rhizome, leaf and seed, respectively (Fig. 3A). Based on aqueous extract data, rhizome and leaf

(A) (B)

Figure 1: *Angelica glauca* (A) and *Dioscorea deltoidea* (B) and their dried roots and rhizome, respectively.

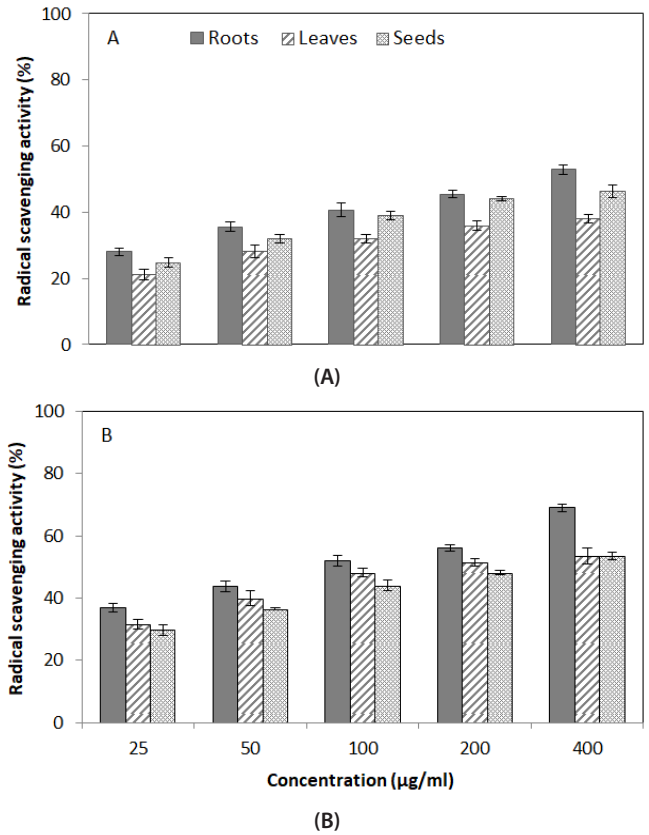

Figure 2: DPPH radical scavenging activity of residual extracts of various plant parts (roots, leaves and seeds) of *A. glauca* in water (A) and methanol (B). Data are means ± SE of triplicate determinations.

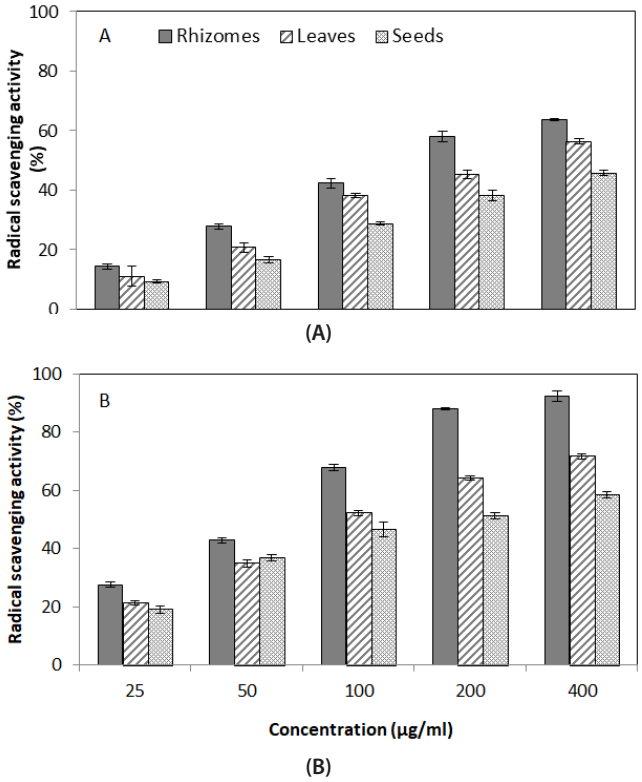

Figure 3: DPPH radical scavenging activity of residual extracts of various plant parts (rhizomes, leaves and seeds) of *D. deltoidea* in water (A) and methanol (B). Data are presented as mean ± SE of triplicate determinations.

extracts possessed comparable antioxidative activity that was greater than that observed in case of seed extract. Similarly, at same range of concentrations in methanolic extracts, the radical scavenging activity observed was between 28-92, 21-72 and 19-59% in rhizome, leaf and seed, respectively (Fig. 3B). In this case, rhizome extract showed highest antioxidative activity that was followed by leaf and seed extract, respectively.

Pyrogallol autoxidation assay

The pyrogallol autoxidation assay reveals the superoxide anion scavenging activity. It was progressively inhibited with increasing concentrations of both aqueous and methanolic extracts of *A. glauca* and *D. deltoidea*. In *A. glauca,* at 25 µg/ml aqueous extract of root, leaf and seed, the superoxide anion scavenging ability was 13, 6 and 11 % that increased to 52, 31 and 46, respectively with an increase in extract concentration to 400 µg/ml (Fig. 4A). In methanolic extracts of root, leaf and seed the values were 24, 12 and 16 % (at 25 µg/ml) and 61, 43 and 50% (at 400 µg/ml), respectively (Fig. 4B). The methanolic extracts were only marginally better in efficacy.

In *D. deltoidea*, the superoxide scavenging ability of the aqueous extract of rhizome, leaf and seed was found to be 12, 8 and 7% (at 25 µg/ml) and 44, 29 and 32% (at 400 µg/ml), respectively (Fig 5A). For methanolic extract, these values were 32, 15 and 14% (at 25 µg/ml) and 69, 43 and 40% (at 400 µg/ml), respectively (Fig. 5B). Thus, a superior superoxide anion scavenging ability was observed in methanolic extract of *D. deltoidea*, compared to the corresponding aqueous extract concentrations (Fig. 5).

Discussion

Plants synthesize numerous phytochemicals belonging to phenolic compounds, terpenoids and alkaloids. Many of them exhibit strong antioxidative properties and delay or prevent oxidative damage (Halliwell and Gutteridge 2007). They also serve as defense molecules against pests, micro-organisms, herbivores and harsh climatic conditions. Medicinal plants comparatively contain high amount of such molecules or antioxidants and thus show antiaging, anti-inflammatory and anticancerous properties (Xu *et al*. 2017). A large number of medicinal plants have been investigated for their antioxidant potential (Krishnaiah *et al.* 2011; Škrovánková *et al.* 2012; Sharma *et al.* 2013) and many of them were found to be highly effective against oxidative stress. Several synthetic antioxidants (butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhy-droquinone (TBHQ), propyl gallate, etc.) are known to exhibit higher antioxidative activities than the natural ones (Pokorný 2007). However, the natural antioxidants are generally preferred over synthetic ones, because the former are known to have better compatibility with human

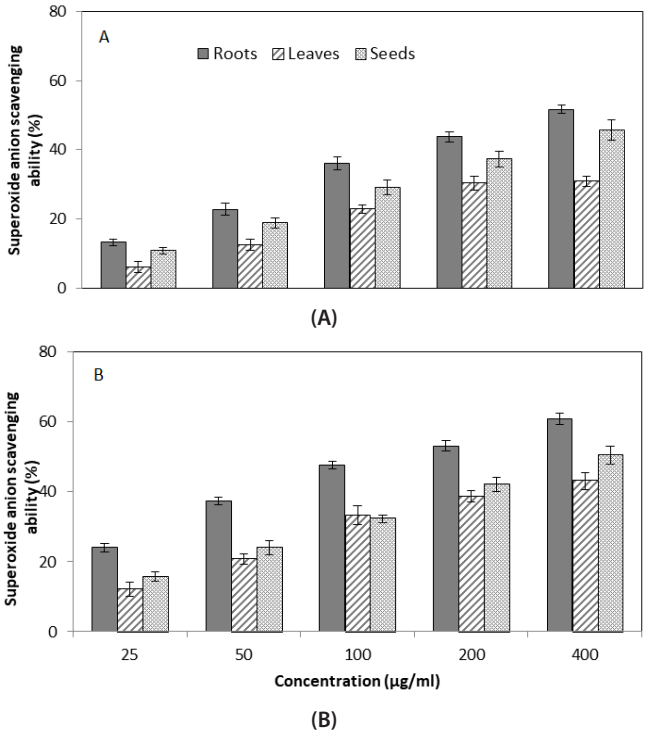

Figure 4: Superoxide anion scavenging ability of residual extracts of various plant parts (roots, leaves and seeds) of *A. glauca* in water (A) and methanol (B). Data are presented as mean ± SE of triplicate determinations.

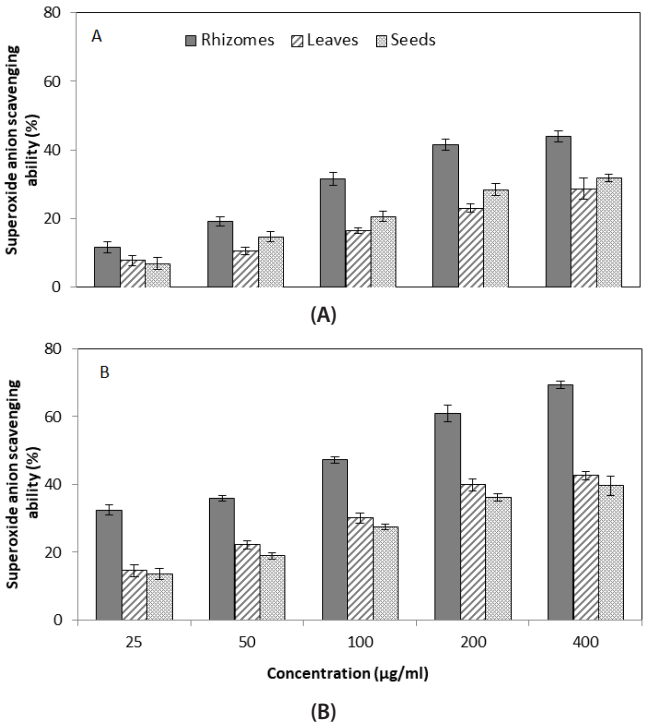

Figure 5: Superoxide anion scavenging abiliity of residual extracts of various plant parts (rhizomes, leaves and seeds) of *D. deltoidea* in water (A) and methanol (B). Data are presented as mean ± SE of triplicate determinations.

body and thus exhibit lesser toxicity and/or no known side effects (Shebis *et al.* 2013). In contrast, synthetic antioxidants may prove harmful if consumed in high dosages for long period of time (Kahl and Kappus 1993). We analyzed the antioxidant activity of different plant parts (seeds, leaves and roots/rhizome) of two highly important medicinal plants namely, *Angelica glauca* and *Dioscorea deltoidea* using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity and pyrogallol autoxidation assay.

In present study, the DPPH assay revealed substantial scavenging (antioxidative) effects of both aqueous and methanolic residual extracts of different plant parts of *A. glauca* and *D. deltoidea*. The effect was concentration dependent across the extract of different plant parts of *A. glauca* as well as *D. deltoidea* (Fig. 2, 3). The antioxidative properties of different plant parts of *A. glauca* have also been reported previously (Joshi *et al.* 2008; Irshad *et al.* 2011; Arya and Mehta 2017, Nengroo and rauf 2021). Irshad *et al.* (2011) observed 93% DPPH radical scavenging activity and 45% inhibition of peroxidation due to *A. glauca* essential oil, showing immense role as natural antioxidant as well as to protect oxidative deterioration in food products. Rawat and Gupta (2017) reported 27 to 58% DPPH activity in methanolic extract of the seeds of *A. glauca* collected from different locations in Uttarakhand. Seeds collected from higher altitudes exhibited relatively higher levels of phenols and flavonoids and as a result showed higher antioxidative activity than those collected from lower altitudes. They established a positive correlation between DPPH activity, altitude variation and total phenol, flavonoid contents. Dudonne *et al.* (2009) and Riaz *et al.* (2020) also observed similar correlation between free radicals (DPPH) scavenging efficacy and phytochemical (phenolic and flavonoids) contents. The observations emphasize the significance of high-altitude medicinal plants and the role of these molecules as potential natural antioxidants. In *D. deltoidea*, rhizome extract showed highest antioxidative activity that was followed by seed and leaf extract, respectively. Amir *et al.* (2011) revealed strong antioxidant activity in aqueous extract of *D. deltoidea* rhizomes that surpassed the positive control, ascorbic acid. Similarly, Bhandari and Kawabata (2004) and Barman *et al.* (2018) also reported significant DPPH radical scavenging ability of *D. deltoidea* rhizomes in methanolic extracts. Waris *et al.* (2022) recorded 30-85% DPPH activity due to 200-1000 µg/ml of *D. deltoidea*. The free radical scavenging property of the extracts of different plant parts of *A. glauca* and *D. deltoidea* was also monitored through pyrogallol autoxidation assay that reveals the superoxide anion scavenging activity. The pyrogallol autoxidation was progressively inhibited with increasing concentrations of both aqueous and methanolic extracts of *A. glauca* and *D. deltoidea* (Fig. 4 and 5).

The antioxidative and radical scavenging activity was found to be invariably greater in case of the methanolic extracts as compared to the aqueous extracts at all the tested concentrations. Arya *et al.* (2021) also found highest antioxidant potential in methanolic extract of *A. glauca* as compared to aqueous and other solvent extracts. Similarly, Jamwal *et al.* (2016) also recorded high antiradical activity in methanolic extract than aqueous extracts. In contrast, Gupta and Singh (2018) and Singh and Patra (2018) found higher antiradical activities in aqueous extracts than methanol extracts in *Berberis* and *Polygonatum* species, respectively. Sabir *et al.* (2013) revealed highest % DPPH inhibition in petroleum ether extract (82%) followed by chloroform (57.1%), methanol (49.7%) and n-butanol (7.5%). Arya and Mehta (2017) reported 92-96, 30-70 and 40-70% DPPH activity in methanolic, petroleum ether and aqueous extract, respectively due to 100-400 µg/ml concentrations of *A. glauca* roots. Borah *et al.* (2012) revealed higher superoxide radical scavenging activity in ethanol followed by methanol and acetone extracts in *Oxalis corniculate*. The solvent dependent variation in antioxidative and radical scavenging activity clearly indicate the importance of solvent selection for optimum extraction and better activity. The quantitative differences in antioxidative properties observed in current study and those reported earlier could in part be ascribed to the growth conditions prevailing in the location where the plants were collected from. They could also be due to methodological variations.

Further, in most of the results obtained, the concentration dependent effect was more pronounced at lower (25 to 100 µg/ml) than at higher concentrations (200, 400 µg/ml) of extracts. For example, a 40% jump in antioxidative activity in methanolic rhizome extract of *D. deltoidea* was observed due to increase in concentration from 25 µg/ml to 100 µg/ml. In contrast, only a 4.5% increase was observed when the concentration increased from 200 µg/ml to 400 µg/ml (Fig. 3B). Bhatt *et al.* (2018) also observed such trend with *Berberis* species. Many bioactive compounds/secondary metabolites have been isolated from different plant parts of *A. glauca* and *D. deltoidea* by several workers (Chandra *et al*. 2013; Abbasi *et al*. 2015; Akalya and Subasri 2016; Naseem *et al.* 2024) and many of them reported to act as natural antioxidants and/or capable of scavenging free radicals (Kim *et al.* 2003; Kukic *et al.* 2006). Although we did not measure any of the bioactive molecule in present study but, the antioxidative properties observed can be ascribed to these compounds present in different parts of the plants.

Conclusion

The DPPH and pyrogallol autoxidation assay revealed strong antioxidative properties in different plant parts of two important medicinal plant species, *A. glauca* and *D. deltoidea* from Kinnaur, Himachal Pradesh. In both, *A. glauca* and *D. deltoidea*, a comparison of different plant parts with regard to the antioxidative potential revealed a higher activity in the roots/rhizomes as compared to seeds and leaves across the tested extract concentrations. Further, methanolic extracts exhibited higher activity as compared to the corresponding aqueous extracts in both species. The antioxidative properties could apparently be ascribed to diverse secondary metabolites reported in both the species.

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