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Antioxidant Potential of *Angelica glauca* of Uttarakhand Region

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Abstract: In the present work, the effect of altitudinal variations on the total phenol, flavonoid, and antioxidant potential of seeds of *Angelica glauca* collected from 17 different locations of Uttarakhand has been investigated. Methanolic and acetonic extracts of seed showed significantly increasing amounts of total polyphenol content, and antioxidant potential with rising altitude. Methanolic extract of seed were found to have significantly greater polyphenol, and antioxidant potential as compared to acetonic extract. The methanolic seed extract of Bageshwar (3,353 m) showed the maximum total phenolic content 1,521 µg gallic acid equivalent/ 50 mg of dry weight. The methanolic seed extract of Bageshwar showed the maximum antioxidant activity in β-carotene (77.7 %) and DPPH activity (37.4 %) also. The results of this study exhibited good correlation with total polyphenol and antioxidant potential in all the samples followed by the increasing tendency towards rising altitude.

Key words: *Angelica glauca,* altitudinal variation, antioxidant potential, polyphenol, β-carotene.

Introduction

Plants are one of the most important sources of medicines. Today mostly drugs are derived from plants. The medicinal plants are rich in secondary metabolites (which have potential sources of drugs) and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability [1]. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice. One possible reason for this is the perception of them having lesser side effects [2]. With recent advances in medical and nutrition sciences, natural products and health promoting foods have received extensive attention from both health professionals and the common population [3]. In India, plant species are known to have medicinal value which results for the combination present in these plants [4]. The medicinal plants in Uttarakhand are lists well over 700 species. About 85 % of the rural North Indian people population still used traditional herbal remedies utilizing the local plant resources. The role of medicinal plant in disease prevention or control has been attributed to the anti-oxidant properties of their constituent such as vitamins, terpenoids, phenolic acids, tannins, flavonoids, quniones, coumarins, alkaloids, which are rich in antioxidant activity [5].

Angelica glauca (family: apiaceae) commonly known as choru, gandhrayan being native and endemic of Himalyan region, is distributed along 2000 to 3,900 m in Uttarakhand, Jammu &Kashmir Himachal Pradesh. *A.glauca* (Chora) is a tall, erect perennial glabrous aromatic herb that grows

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to a height of about 1-1.5 m. The leaves are pinnately divided. The flowers are present in large stalked, highly branched umbels and are mostly white, purple and yellow in colour. The species is well known for its aromatic as well as medicinal values. The essential oils (1.3 %) yielding roots contain valeric acid, angelic acid and angelisine resin. The roots are used for stomach and urinary disorders mostly in constipation and gastric problems, rheumatism and bronchitis [6] and considered cardio active [7]. Local communities use its roots as a spice especially for seasoning curry. Roots and seeds of this species are used as carminative, diaphoretic, diuretic, antiseptic and antidepressant (on central nervous system). Roots are also used in the treatment of leucoderma and for dental preparation [7].

Earlier investigations on native as well as cultivated plants showed significant positive correlations with the mean above sea level of the growing site and the amounts of certain phenolic antioxidants [8]. It is known from the previous studies that, both the content and the activity of compounds that are part of their antioxidant defense system vary with the change in altitude [9]. These variations with the altitude is assumed to be associated with the enzymes involved in the synthesis of phenolics, found to be produced in greater quantities or show increased activity at low temperature [10]. In addition to this, low temperatures have also been shown to enhance synthesis of phenylalanine ammonia lyase (PAL) in plants leading to increased production of phenolics, including flavonoids [10,31]. It is also noticed that, the decreased temperatures trigger greater biosynthesis of phenolics in some plant species, even in the absence of UV-B radiation [11]. Solar UV radiation increases with elevation and with proximity to the sea. Quantitatively, it is known that UV-B (280-315 nm) increases 14-18 % per 1,000 m rises in elevation. Therefore, with greater UV radiation in higher altitudes, the plants are expected to show better antioxidant properties.

The occurrence of *Angelica glauca* over a wide range of altitudinal zones makes it a preferable species for studying variations of its secondary metabolite growing under different altitudinal conditions. Moreover, there is only limited information about the altitudinal relationship of secondary metabolite and antioxidant activity in *Angelica glauca* seeds. In this study, we aim to investigate the antioxidant activity of *Angelica glauca* with respect to their altitudinal variation. The present investigations may help in attaining maximum medicinal value from *Angelica glauca*. The correlation between antioxidant capacity and phenolic content of seed samples collected from different altitudes was also determined. These kinds of studies have been performed on a few plants [12,13]. However, to the best of our knowledge, no such studies have been performed previously on *Angelica glauca* and hence the present study may help enormously in exploiting medicinal potential of these species.

Materials and Method

Chemicals

All chemicals that were used in this study (mentioned in subsequent sections) were manufactured by Sigma-Aldrich (St. Louis, MO, USA), and Merck (Darmstadt, Germany).

Seed samples

Seeds of *Angelica glauca* were collected from 17 different altitudinal geographic regions of Uttarakhand, India as listed in **Table 1** during October-November 2012. Immediately after the collection, seeds were dried at room temperature (20±5ºC) for 1 week and stored at 4ºC temperature.

Preparation of seed extracts

The seed samples were milled with a mortar and pestle. Seed samples of different geographic regions (1 g each) were extracted with 20 mL of 80 % methanol and acetone containing 2 % (v/v) HCL, and kept on shaker for 24 hr at 1,000 rpm followed by centrifugation at 2,655 × g for 10 min at room temperature. The supernatant was collected and stored at “20ºC until analysis.

Total phenolic content (TPC) estimation

TPC of different extract was assayed using the Folin-Ciocalteu reagent. The reaction mixture contains 50 µL of sample extract, 1.58 mL deionized water and 100 µL of Folin Ciocalteu reagent well

mixed together. This mixture is subjected to incubation for 2 min followed by addition of 500 µL (20 %) sodium carbonate solutions. Subsequently, the reaction mixture was left for 1 hr and the absorption spectra of each sample were recorded at 765 nm wavelength using a Lambda-2 Spectrophotometer (Perkin-Elmer,Waltham, MA, USA). All the experiments were conducted in triplicates and TPC of samples were expressed as µg gallic acid equivalents (GAE)/mg of dry weight (d.w.) through the calibration curve with gallic acid. The calibration curve range was 50-400 µg/mL (r=0.991).

Total flavonoid estimation

The total flavonoid content (TFC) was determined using a colorimetric method described by Marckam [14] with slight modifications. Briefly, the extracts of each sample (100 µL) were appropriately diluted and were added with 75 µL of 5 % $NaNO_2$ solution followed by the addition of 150 µL of 4 % freshly prepared $AlCl_3$ solution and 0.5 mL of 1 M NaOH solution. Finally, 1 mL of distilled water was added and the reaction mixture was incubated for 5 min. Subsequently, the absorption spectrum of the mixture was measured at 510 nm wavelength. Three replications/treatment were analyzed and the results were expressed as ìg rutin equivalents (rutin)/mg d.w. through the calibration curve with rutin. The calibration curve range was 10-90 µg/mL (r=0.994).

Free radical scavanging activity by 1,1-diphenyl-2- picrylhydrazyl (DPPH) assay

Hydrogen atom or electron donation ability of the corresponding extracts was measured by bleaching of the purple-colored methanol solution of DPPH. Reduction of DPPH by an antioxidant or by antiradical species results in a decrease of absorbance at 517 nm. The total antioxidant activity (TAA) was estimated by the Trolox equivalent antioxidant capacity (TEAC) assay [15]. For this, 0.1 mL of the sample extract was added to 0.9 mL methanolic solution of DPPH radical (33 mM) followed by vigorous quivering of the mixture for 1 min at room temperature and the absorbance for the sample was measured at 517 nm wavelength against blank. The reaction of DPPH with Trolox was used to compare the radical scavenging activity of a compound to those of Trolox, a water soluble vitamin E analogue. Trolox solution in the 0-1,000 µM concentration ranges were employed for calibration.

β-Carotene bleaching activity

The antioxidant activity of extracts were evaluated according to the β-carotene linoleate model system based on the procedure suggested by Marco [16] and Velioglu *et al.* [17]. Two mL of solution of β-carotene in chloroform (5 mg/mL) were pipetted into a flask containing 200 µL of linoleic acid and 400 mg of Tween 40. The chloroform was removed under vacuum at 45ºC and 100 mL of distilled water were then slowly added to the semisolid residue with vigorous agitation to form an emulsion. Finally, 5 mL of the emulsion was mixed in a tube containing 0.2 mL extract of the samples (50 mg/mL) n concentration followed by measurement of absorbance at 470 nm wavelength. The tubes were placed in a water bath at 50ºC and measurement was conducted at 15 min interval up to 120 min. These determinations were carried out in triplicates. Antioxidant activity was estimated by 3 different methods given by Velioglu *et al.* [17].

Statistical analysis

A detailed statistical analysis was performed with all the data expressed as mean ± standard deviation (SD) of triplicate measurements. Two-way analysis of variance (ANOVA) was carried out to examine the differences between various quantities when different solvents were used. Statistical comparisons between variables (e.g., TPC, TFC, and antioxidant activity with both methods) were performed with Student's t-test. Correlation between the antioxidant activity and TPC was carried out using the correlation in the MS-EXCEL programme.

Results and Discussion

Total phenolic content (TPC)

The sample collection sites and their altitudes are given in **Table 1**. The phenolic compounds may contribute directly to antioxidative action necessitating the investigation of TPC. TPC of all

Table 1. Sampling locations, sample codes and altitudes of Angelica glauca seeds studied

No.	Sampling locations	Sample codes	Altitude (m)
1.	Almora	ALG	1646
2.	Khati	KHG	2210
3.	Phata	PHG	2387
4.	Chamoli	CHG	2600
5.	Mangoli	MLG	3000
6.	Rudranath	RDG	3000
7.	Gangotri	GAG	3100
8.	Badrinath	BNG	3100
9.	Hudu	HUG	3289
10.	Yamunotri	YMG	3293
11.	Bageshwar	BGG	3353
12.	Panwali Kantha	PWG	3300
13.	Dayara	DYG	3400
14.	Mukba	MKG	3600
15.	Mana	MNG	3650
16.	Kedarnath	KDG	3670
17.	Purkhiya	PKG	3900

the samples displayed significant differences ($p<0.001$) as a function of altitude, solvents and locations. A noteworthy ($p<0.001$) solvent effect was observed due to difference in the TPC in methanol, acetone seed extracts. The TPC of seed and of methanolic and acetonic extract showed the notable ($p<0.001$) variation on the basis of the altitudinal range. The TPC of seed of methanolic extract ranged from 388.5 to 1,521.2 µg GAE/50 mg of d.w., (Fig. 1). Similarly, the TPC of seed of acetonic extract ranged from 296.1 to 1978.2 µg GAE/50 mg of d.w., (Fig. 2) respectively. TPC of methanolic extract was found to be significantly ($p<0.001$) higher as compared to that of acetonic extract. These results are in agreement with previously reported works where methanol was found to be the most suitable solvent in the extraction of phenolic compounds from plant tissues due to its ability to inhibit the action of polyphenol oxidases that cause the oxidation of phenolic compounds and its ease of evaporation compared with water [18]. In addition, the yield of extracts also increases with increasing polarity of the solvents [19]. As far as the collection sites were concerned, methanolic seed extract of samples collected from BGG (3,353 m) revealed the highest level of TPC with 1,521 µg GAE/50 mg of d.w., respectively. Similarly, the maximum phenolic content of acetone seed extract of sampled gather from the same location was estimated to be 1978.2 µg GAE/50 mg of d.w.. TPC of methaolic, extract of seed collected from ALG (1646 m) was observed to be minimum i.e., 387.4 µg GAE/50 mg of d.w. Similarly, the acetonic extract of ALG showed the lowest TPC in seed (296.1 µg GAE/50 mg of d.w.). Therefore, it may be concluded that TPC contents of the samples collected from higher and lower altitudes reveal significant variations.

One more notable observation is that the samples collected from locations above the BGG (3,353 m) i.e., samples explored from PWG (3,300 m), DYG (3,400 m), MKG (3,600 m), MNG (3,650m) landraces displayed a slight changeability in TPC with altitude. Quantitatively, the TPC of samples from PWG, DYG, MKG and MNG were estimated to be 1,286, 1,273, 1,189, 1,081 µg GAE/50mg of d.w., respectively. These samples have shown the decreasing tendency towards higher altitude. This behavior might be attributed to the strong effect

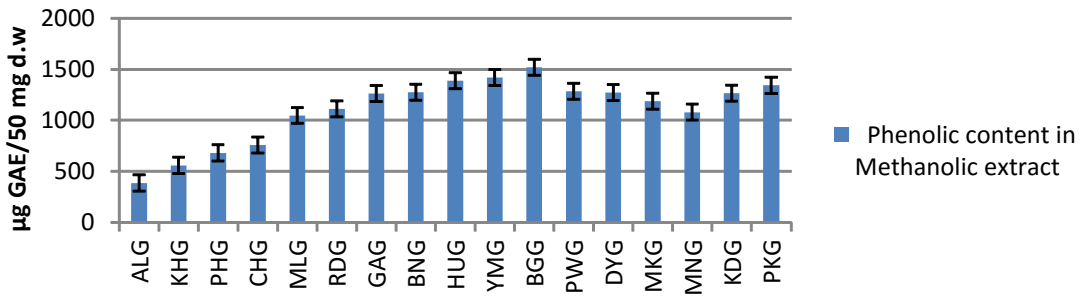

Fig. 1. Total phenolic content of *Angelica glauca* seed in methanolic extract

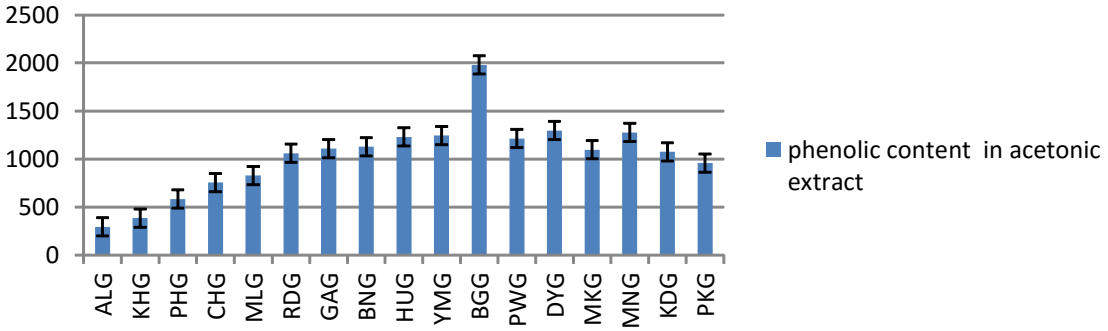

Fig. 2. Total phenolic content of *Angelica glauca* seed in acetonic extract

of temperature on TPC. This argument is well supported from a published work of Wang and Zheng [20], showing that environmental temperature strongly alters antioxidant properties in strawberry. The results of this study, that the variation of TPC with altitude is supported by one of the prevailing theories, which suggest that altitudinal variations may be interpreted as adaptive responses to the elevated level of harmful UVB radiation present at higher altitudes [21]. The report published by Sanders [22] describes that an increase in unsaturated fatty acids is generally associated with cooler climates leading to production of antioxidants for a selfdefense system against environmental stress. This supports our present conclusions suggesting pronounced effects of environmental temperature on TPC. Phenolic compounds play a vital role in the hydrogen peroxide scavenging system of plants, which besides phenolics, contains peroxidase, ascorbic acid, and glutathione. This system functions less efficiently at low temperatures, and more phenolics have to be produced to prevent damage to plants grown at lower temperatures [11]. Recently, Albert *et al.* [23] reported lower air temperature rather than enhanced UV-B radiation as the key factor influencing the altitudinal variation of phenolics in Arnica montana. The effect of change of temperature with altitude on TPC content was also observed by different workers [9,12,13].

Total flavonoid content (TFC)

In the present study, the samples collected from various locations of different altitudes showed slight differences in TFC. The altitudinal effects on TFC were found to be different from that observed for TPC. In the study, it was seen that there was no positive significant correlation of TFC with altitude but there was a difference in TFC of acetonic and methanolic extract of seed. TFC in methanolic and acetonic seed extract ranged from 212.3 to 272.4 and 115.2 to 269.4 µg rutin/50 mg of d.w., (Fig. 3 and 4) respectively. TFC was found to be maximum in the methanolic seed extract of GAG (3,100 m) i.e., 272.4 µg rutin/50 mg of d.w. but in the acetonic seed extract BGG (3,353m) showed the maximum TFC of 269.4 µg rutin/50 mg of d.w.. The minimum TFC in methanolic and acetonic extract of seed sample was found in KHG (2,210 m) and ALG (1,646 m) which was 204.4 and 115.3 µg rutin/50 mg of d.w., respectively. The solvents used for the extraction showed varia-

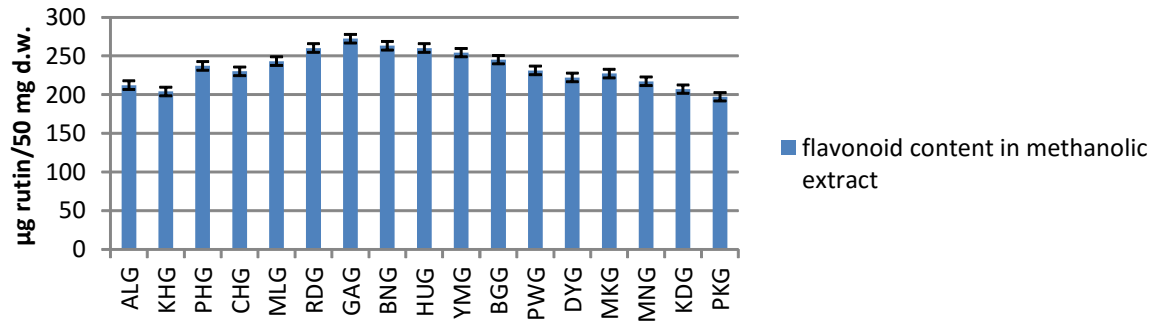

Fig. 3. Total flavonoid content of *Angelica glauca* seed in methanolic extract

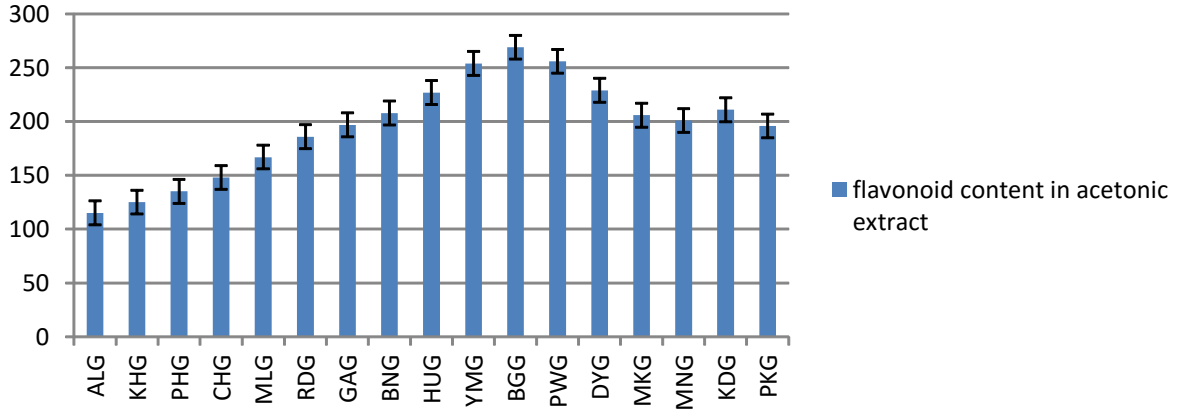

Fig. 4. Total flavonoid content of *Angelica glauca* seed in acetonic extract

tion in TFC for all the samples but without much significance. In our study, this kind of unclear relationship between the TFC and altitude may be explained with the help of previous work, which reported that, the TFC did not always show significant positive correlation with altitude of the growing site, in any cultivating season due to uncontrolled environmental temperature. The results observed in the present work has been supported by the study on flowering heads of *A. montana* cv. ARBO (Asteraceae) and Colombian Amazonian plants that revealed positive correlation with altitudinal variation of TPC but not that with TFC [24]. It was also found that high phenol content was not always accompanied by high flavonoid concentrations [22,23]. It is also established that there is a weak association between TFC and antioxidant activity with a non significant correlation between TFC and antioxidant activity in *A. glauca* [26]. However, the antioxidant function of flavonoids in plants remains unclear and is still being debated [27].

Antioxidant activity

For the estimation of antioxidant potential, we combined 2 complementary techniques based on β-carotene and scavenging of the DPPH radical. We chosen to follow this method as the interpretation of results by a single method can only give reductive suggestion of antioxidant properties of the extracts. A comparison was made between the total antioxidant potential of seed samples of *Angelica glauca* collected from different altitudinal range. The antioxidant potential of the seed extracts were determined by β-carotene and DPPH assays. Significant variations in antioxidant potential by β-carotene assay were observed with respect to solvent system and geographic locations of different altitudes. This method was used to evaluate the antioxidant potential in the methanolic and acetonic seed extracts of different landraces. The values of antioxidant potential significantly varied from high altitude to low altitude. On the basis of this assay, all the extracts of seed, collected from different altitudinal regions exhibited the antioxidant potential. Methanolic extract of seed samples of high altitude showed significantly higher antioxidant potential as compared to that estimated in the samples collected

from lower altitudes. It was also observed that antioxidant potential of methanolic seed extract was notably higher than that obtained from acetonic extract. Antioxidant potential of all the samples based on the inhibition of β-carotene bleaching (antioxidant activity expressed as percentage inhibition).The antioxidant activity of methanolic and acetonic seed extract varied from 61.8 to 77.3 and 40.9 to 75.6 %, (Fig. 5 and 6) respectively. Among all the samples the methanolic seed extract showed a highest antioxidant activity ($p<0.001$) of 77.3 % for BGG (3,353 m) and lowest antioxidant activity of 61.8 % for ALG (1,646 m), which correlates with the TPC. In acetonic seed extract, BGG and ALG also had a maximum and minimum antioxidant activity values.

In acetonic seed extract BGG showed 75.6.2 % and ALG 40.9 % antioxidant activity, respectively, which is significantly ($p<0.001$) less than the methanolic extract. It was observed earlier that a change in solvent polarity alters the ability to dissolve a selected group of antioxidant compounds and influences activity estimation [28]. Antioxidant activity of methanolic The overall order of antioxidant activity for both the extracts was the same for a particular geographic location and altitude, suggesting marked influence of altitude and temperature on the antioxidant activity of seed of *Angelica glauca.* Samples above than BGG followed similar tendency for antioxidant activity as in TPC, this tendency also showed the strong effect of temperature on antioxidant activity. Furthermore, our observation showed pronounced effect of environmental temperature on antioxidant activity, which is supported by earlier investigations on peanut [22], A. montana [24], Colombian Amazonian plants [24,25,30] and Moringa oleifera [29]. It is because of these reason eastern practitioners generally believe that plants from hilly areas are more important from a medicinal and nutritional point of view. The results also confirm that changes in the quantity of phenolic compounds in seed samples have an impact on antioxidant activity of extracts derived from these seed. Moreover, we demonstrated that altitudinal effects on antioxidant activity are not caused by primarily changes in other factor at the growing sites. The

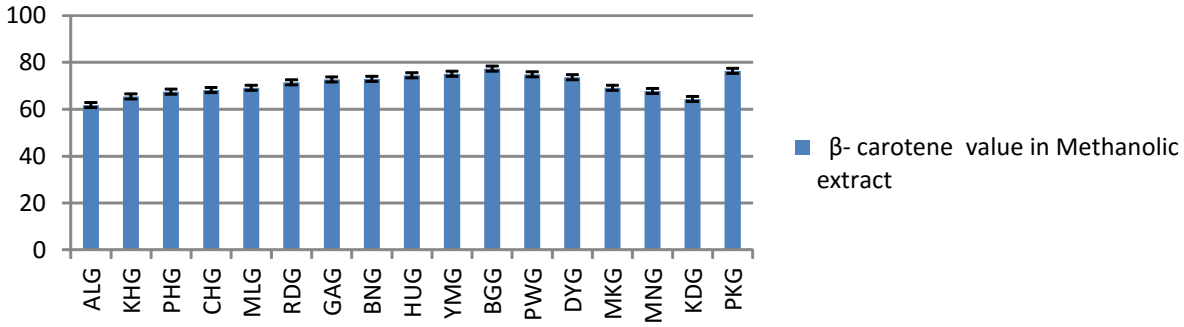

Fig. 5. β-Carotene value of *Angelica glauca* seed in methanolic extract

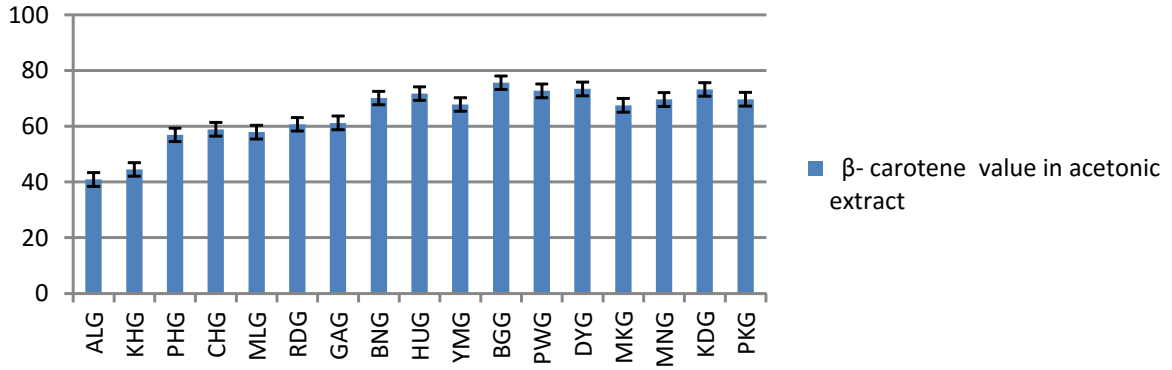

Fig. 6. β-Carotene value of *Angelica glauca* seed in acetonic extract

values were shown the significant ($p<0.001$) variations among the different altitudes.It was observed that all the extracts exhibited an appreciable scavenging activity. Significant ($p<0.05$) differences in scavenging activity were noticed among samples from different altitudes and solvents. Scavenging activity was considerably affected by the variation in altitudes for all the samples [31]. The free radical scavenging activity of extracts of seed samples of *Angelica glauca* were tested by measuring their ability to quench the stable DPPH (Fig. 7 and 8). This assay provides stiochiometric information with respect to the number of electrons taken up by the tested compounds in the presence of the stable free radical. The methanolic extract of seeds collected from BGG showed highest ($p<0.05$) antioxidant activity of 37.4 %.The methanolic extract of ALG showed the lowest activity of 24.7 %. On the other hand acetonic seed extract of samples collected from BGG exhibited highest antioxidant activity (58.4 %) and ALG showed lowest activity of 27.2 %. The present investigations revealed that the DPPH radical scavenging potential of seed samples from plants grown at higher altitudes was significantly higher than that noticed in the case of plants cultivated at lower altitudes. This result was not surprising as polyphenol, whose amounts were also positively correlated with altitude, are potent radical scavengers [15,31]. The result is nonetheless noteworthy as other compounds, which are present in extracts are also contributing to the overall antioxidant potential of the extracts. The methanolic and acetonic extract of seeds have shown almost similar trend in their antioxidant potential for both the methods.

Conclusion

To conclude, in this study, the effect of variation of altitude on the total phenol and flavonoid content of seeds of *Angelica glauca* along with associated antioxidant potential was investigated. It was observed that the extracting solvent significantly affected the total polyphenol and anti-

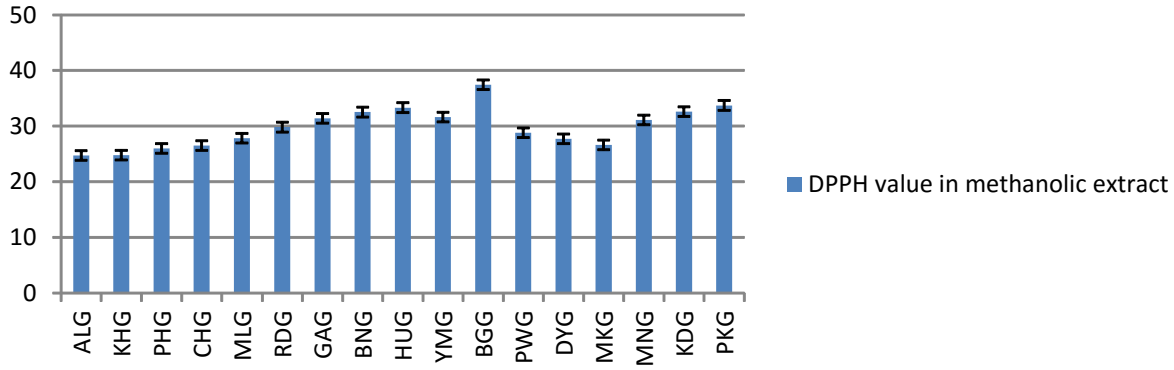

Fig. 7. DPPH value of *Angelica glauca* seed in methanolic extract

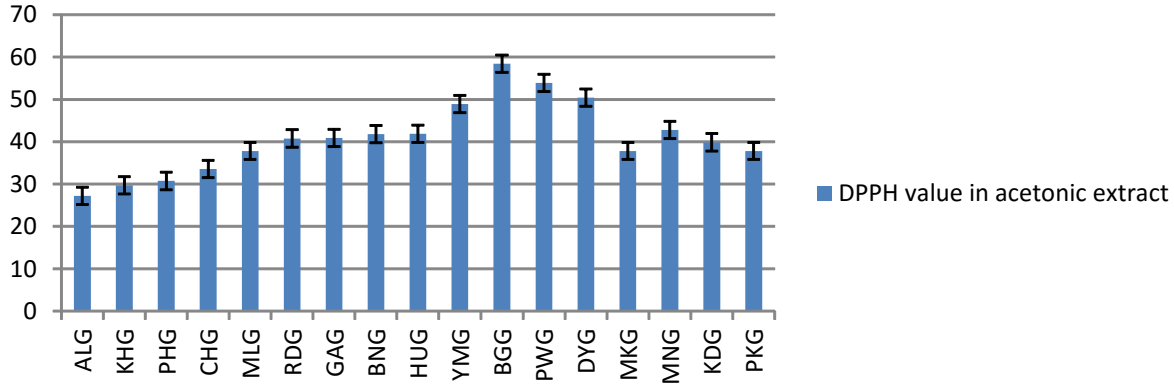

Fig. 8. DPPH value of *Angelica glauca* seed in methanolic extract

oxidant potential of extracts of seed of various altitudes. It was conclusively shown that extracts with higher antioxidant capacity had higher polyphenol contents also.

In the present work, it could be convincingly seen that solvent and altitudinal variations have profound effects on the polyphenol content, antioxidant activity of all the samples of *Angelica glauca*. However, TFC had not shown any significant effect of altitudinal variation. All the measured parameters of samples from high altitudes were relatively higher than those collected from lower altitudes. The outcome this study also suggested that environmental temperature plays a significant effect on all the measured parameters except TFC.

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