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Evaluation of Phyto-Chemical, Biochemical and *In-Vitro* Antioxidant Potential of *Angelica Glauca* Grown at High Altitude Areas of Western Himalayas

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ABSTRACT

Angelica glauca Edgew is an important medicinal and aromatic herb (family Apiaceae). The roots of *A. glauca* commonly used as spices by local peoples. In the present study, the phytochemical constituents, biochemical parameters and, *in-vitro* antioxidant activity of *A. glauca* roots collected from the Himalayan region have been studied. For preliminary phytochemical analysis, the hydroalcoholic and aqueous root extract of *Angelica glauca* were screened for the presence of carbohydrates, protein, alkaloids, glycosides, sterols, triterpenes, saponin, tannins, phenols, flavonoids, and coumarin. The biochemical parameter and *in-vitro* antioxidant potential of *Angelica glauca* were analysed by using standard methods. Phytochemical analysis of extract showed major classes of phytochemicals constituents such as carbohydrates, protein, alkaloids, glycosides, sterols, triterpenes, saponin, tannins, phenols, flavonoids, and coumarin. The results from the current study demonstrated that *A.glauca* roots contained carbohydrate (21±0.72 %), crude protein (12.7±0.31 %), total ash (3.86±0.034 %), dietary fiber (18.9±0.14 %), total fat (4.5±0.38 %) and ascorbic acid (68.5±0.19mg/100g). The hydroalcoholic extract showed the highest quantity of total phenol, total flavonoids, and total tannin content compared to aqueous extract. The hydroalcoholic extract exhibited high DPPH radical scavenging activity (IC_{50}=68.1±0.34 µg/ml). The result showed that *A.glauca* roots have high nutritional and antioxidant potential. Hence the plant can be used as a nutraceutical and natural antioxidant.

Keywords: Biochemical; Phytochemical; *A.glauca*; Antioxidant

1. INTRODUCTION

Nowadays, people are more attracted to spices due to nutraceutical and antioxidant potential[1-2]. Spices are an essential part of the food system throughout the world, mainly used for enhancing taste. The spices are capable of increasing the nutraceutical value of the prepared food due to several types of phytochemicals[3]. Several spices such as clove, cumin, cinnamon, curcumin, ajwain, fenugreek seed, and thyme, etc., can treat infectious diseases due to the presence of various types of secondary metabolites. These properties have proposed a new way of the utilisation of these spices as nutraceuticals[4].

The dried roots of Angelica glauca used as spices by local peoples. Angelica glauca Edgew. (family Apiaceae) is a medicinal and aromatic herb of Western Himalayas[5-6]. A. glauca, locally called Choru or Gandhrayan, being native and endemic of the high altitude Himalayan regions plant is distributed in Uttarakhand, Jammu and Kashmir, and Himachal Pradesh[6]. The roots used for stomach and urinary disorders, mostly in constipation and gastric problems, rheumatism and bronchitis[6]. According to IUCN, Angelica glauca are considered vulnerable and critically endangered due to indiscriminate collection of the plant's materials from wild[7-8]. Still, their propagation protocols are being developed for making this plant available through cultivation practices[9-10]. Earlier studies on this species are mainly paying attention to the estimation of their essential oils[11-13]. A few type of research have been studied on evaluating the nutritional potential and the antioxidant activities of A. glauca[14-15]. Therefore, the present study was conducted to estimate the phytochemical constituents, biochemical parameters, and in-vitro antioxidant potential of A. glauca roots.

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2. METHODOLOGY

2.1 Plant Sample

The roots of Angelica glauca (Apiaceae) were collected from Bathad district Kullu in October 2018. The authentication of the plant was carried out from CSIR-National Botanical Research Institute (NBRI Lucknow) with herbarium accession number (103302). The plant material was air-dried in the shade, ground to a fine powder and packed in a tightly closed container.

2.2 Extraction of Plant Material

The root powder was extracted with hydroalcoholic (70: 30) and aqueous solvent by the cold maceration process with occasional shaking. The extracts were filtered using Whatman No. 1 filter paper. After repeated extraction three times, the

filtered was combined and concentrated by a rotary evaporator. The samples were finally lyophilised for further studies.

2.3 Organoleptic Study

The organoleptic study of a root includes its visual appearance to the naked eye and its characteristics, colour, odour, taste, texture, shape[16].

2.4 Qualitative Phytochemical Analysis

The hydroalcoholic and aqueous extract of A.glauca roots were subjected to qualitative phytochemical screening for its different phytochemical constituents such as carbohydrates, proteins, tannins, phenols, saponin, glycosides, alkaloids, flavonoids, terpenoids, and coumarin according to the standard methods[17].

2.5 Estimation of Biochemical Parameters

The moisture content of roots was determined by using the HE53 Moisture analyser (India). The various parameters like ash values (total ash, acid insoluble ash, water-soluble ash) were carried out according to the standard methods[16]. Quantitative analysis of total carbohydrate content in A.glauca root was done by Anthrone method[18]. The estimation of protein was determined by Lowry's method[19]. Total fats and crude fiber were also estimated according to the reported method[20]. The ascorbic acid content in root was performed by 2, 6-dichlorophenol indophenol method[21].

2.6 Determination of antioxidant phytochemical constituents

The phenol content of extracts was estimated by the Folin–Ciocalteau method with catechol as standard[22]. Tannin was performed by Folin-Denis method using tannic acid as standard[23]. The contents of total flavonoids of extracts were determined by the Aluminium chloride colourimetric method using quercetin as standard[24].

2.7 Determination of Antioxidant Constituent

2.7.1 DPPH Radical Scavenging Assay

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of free radical-scavenging activities. Different aliquots of both extracts were taken in test tubes, and volume was made up to 1 ml with methanol. Then added a 2 ml methanolic solution of DPPH (0.1 mM) in all test tubes. After 30 min., the absorbance was taken at 517 nm. Ascorbic acid was used as standard[25]. Percentage inhibition was calculated as

Per cent radical scavenging activity= $[(Abs_{control} - Abs_{sample}] / Abs_{control} \times 100$

Where,

Abs control = absorbance of DPPH radical with methanol;

Abs sample = absorbance of DPPH radical with sample extract/standard.

2.7.2 Statistical Analysis

The results were recorded from triplicate observations and articulated as mean ± SD.

3. RESULT AND DISCUSSION

3.1 Organoleptic Character

The organoleptic characters of the root were shown in Table 1.

3.2 Qualitative Phytochemical Analysis

The phytochemical screening of the hydroalcoholic and aqueous root extracts showed various phytoconstituents, cited in Table 2. The different phytoconstituents include alkaloids, carbohydrates, glycosides, sterols, terpenoids, saponin, tannin, protein, phenols, flavonoids and coumarin were present in both the extracts. The presence of various phytoconstituents in herbal plants plays a crucial function for good health by preventing disease[26].

Table 1. Organoleptic characters of the roots of *A.glauca*

Organoleptic characters	*A.glauca* root
Colour	Brown
Odour	Aromatic
Taste	Bitter
Shape	Cylindrical
Texture	Horny and hard

Table 2. Phytochemical screening of *A. glauca* roots extract

Tests	*A. glauca*	
Alkaloids	**Hydroalcoholic extract**	**Aqueous extract**
Mayer's Test	+	+
Wagner's Test	+	+
Carbohydrates		
Molisch's Test	++	++
Fehling's Test	++	++
Glycosides		
Keller-killani Test	+	+
Sterols and Triterpenes		
Salkowski's Test	+	+
Saponins		
Foam Test	+	+
Protein		
Biuret Test	++	++
Tannins		
Gelatin Test	+	+
Phenols		
Ferric chloride test	+	+
Flavanoids		
Lead acetate test	+	+
Coumarin	+	+

** + presence ++ moderate presence

3.3 Estimation of Biochemical Parameters

The biochemical parameters such as moisture, total ash, acid insoluble ash, water-soluble ash, carbohydrate, protein, total fat, dietary fiber, and ascorbic acid were estimated in dried root powder A.glauca and results cited in Table 3.

Table 3. Biochemical parameters of *A.glauca* roots (dry weight basis).

Biochemical parameters	*Angelica glauca*
Moisture Content (%)	9.0±0.060
Total Ash (%)	3.86±0.034
Acid Insoluble Ash(%)	1.98±0.012
Water-soluble Ash (%)	1.88±0.017
Fat (%)	4.5±0.38
Fiber (%)	18.9±0.14
Carbohydrate (%)	21±0.72
Protein (%)	12.7±0.31
Ascorbic acid (mg/100g)	68.5±0.19

*Value is expressed as Mean±SD(n=3)

Table 4. Antioxidant constituents in the hydroalcoholic and aqueous root extract of A.glauca

Antioxidant constituents	Hydroalcoholic extract	Aqueous extract
Total Phenol(mg/g)	9.0±0.089	7.4±0.041
Total Flavonoids(mg/g)	6.24±0.008	4.34±0.026
Total tannins(mg/g)	2.1±0.032	1.8±0.017

*Value are expressed as Mean±SD(n=3)

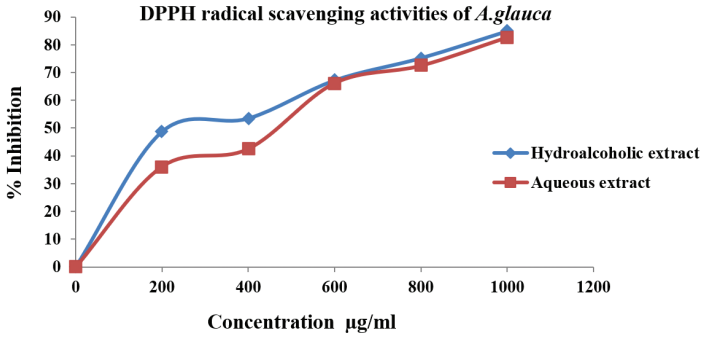

Figure 1. DPPH radical scavenging activities of the extracts of *A.glauca.*

Table 5. IC_{50} values of *A. glauca* root extracts and standard Ascorbic acid in µg/ml against in-vitro DPPH radical system

Extracts/Standard	The IC_{50} value of DPPH (µg/ml) radical scavenging
Hydroalcoholic extract	68.1±0.34
Aqueous extract	[1]88.3±0.001
Ascorbic acid	8.1±0.071

*Values were expressed as mean ± SD (n = 3).

The moisture content was observed at 9 per cent on a dry weight (d.w.) basis. The ash contains inorganic material of the plant, including oxides and salts containing anions such as phosphates, sulfates, chlorides, and other halides and cations such as sodium, potassium, calcium, magnesium, iron, manganese, etc[27]. The result observed that the root has a good source of carbohydrates (21 %) and protein (12.7 %), respectively. The plants, which around 12 per cent caloric values from protein, are considered as a good protein source[28]. Diet rich in fiber aid in digestion, bowel problem, constipation, and colon cancer[29-30]. It was found that root exhibited a high amount of crude fiber of 18.9 per cent. The roots also contain an ample amount of fat (4.5 %). Ascorbic acid is a naturally occurring antioxidant compound found in medicinal plants, vegetables, fruits, and whole grains[31]. The plant roots showed the good quantity of ascorbic acid content (68.5 mg/100g).

In the present study, the values of carbohydrates, protein, and lipid were found higher as compared to reported in previous investigations carbohydrate (29.8mg/g), protein (106.3 mg/g), and lipid (29.8 mg/g) in A .glauca roots[32].

3.4 Determination of antioxidant constituents

The hydroalcoholic and aqueous root extracts of A.glauca were examined for their phenolic, flavonoid, and tannin contents. The results are cited in Table 4. The various secondary metabolites such as phenolics, flavonoids, tannins have pronounced medicinal properties. Phenols are vital plant constituents due to their free radical scavenging ability[33]. The hydroalcoholic extract showed high phenolic content as compared to aqueous extract. Flavonoids have health-promoting properties due to their high antioxidant capacity[34]. The hydroalcoholic extract contains high flavonoids content (6.24 mgQE/g dry extract weight) followed by aqueous extract (4.34 mgQE/g dry extract weight). Tannins are reported as potential antioxidants, and that may reduce the risk of cancer and cardiovascular diseases[35]. The hydroalcoholic extract showed higher tannin content (2.1mg/g dry weight) than aqueous extract (1.8 mg/g dry weight).

3.5 DPPH Radical Scavenging Assay

The scavenging effect of both the extracts (hydroalcoholic and aqueous) on DPPH radical was shown in Fig. 1. The IC_{50} values of scavenging DPPH radicals for the hydroalcoholic and aqueous extract were 68.1±0.34 and 188.3±0.001 µg/ml, respectively (Table 5). The IC_{50} value of standard antioxidant ascorbic acid was found 8.1±0.07 µg/ml. In the present study, the hydroalcoholic extract of A.glauca exhibited better DPPH radical scavenging activity than aqueous extract.

4. CONCLUSION

It can be concluded from the study that the Himalayan medicinal plant Angelica glauca roots contain an appreciable amount of bio-chemical constituents and several phytochemical constituents. Roots are also a good source of antioxidant constituents which are total phenols, flavonoids, and tannins. It has also shown good antioxidant activity. It is further observed that the hydroalcoholic extract of the roots exhibited better antioxidant activity and antioxidant constituents than aqueous

extract. Hence, the roots of the plant can be a good source of nutraceuticals and natural antioxidant.

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