Pak. J. Bot., 52(1): 257-260, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-1(44)

COMPARITIVE PHYTOCHEMICAL AND PHYSICOCHEMICAL STUDY OF SEEDS OF THE GENUS *ANGELICA* L. FROM NEELUM VALLEY AZAD KASHMIR, PAKISTAN

ANJUM PERVEEN*, SHABIR IJAZ AND NAUSHEEN GHAFFAR

Centre for Plant Conservation University of Karachi, Pakistn
**Corresponding author's email: anjum_tahir@hotmail.com*

Abstract

Seeds of *Angelica* species *Angelica glauca* Edgew. and *Angelica archangelica var himalaica* (Clarke) E. Nasir family Umbelliferae were collected for phytochemical and physico-chemical study. Seeds and other parts of the *Angelica* species are used to cure in variety of ailments in many countries. Seeds of two species of the *Angelica* were analyzed for the detection of bioactive compounds by using acetone, chloroform, methanol and water extracts. Maximum solubility of seeds extract of both species was high in water solvent while acetone and chloroform were not ideal solvents for extraction while methanol was a moderate solvent. Chemically the seeds of both *Angelica* species showed variety of bioactive compounds including alkaloids, phenolic compounds and flavonoids thus, seeds can be used for medicinal purposes after ascertaining their pharmacological activity.

Key words: *Angelica glauca*, *A. archangelica* var. *himalaica*, Phytochemical, Physicochemical, Umbelliferae.

Introduction

Phytochemical compounds or secondary metabolites derived from plant and primary source of new drugs discovery (Newman & Cragg, 2012). These compounds are considered as healing agents and useful for curing many diseases in human and animals. According to their function and chemical structure these are categorized into different classes such as alkaloids, carbohydrate, protein, phenolic compounds, glycosides, terpenoids, saponin etc. Alkaloids, terpenoids and phenolic compounds show several important pharmacological activities viz anti-bacterial, anti-viral anti-inflammatory, anticancer, inhibition of cholesterol synthesis and anti-malarial (Mahato & Sen, 1997). Polyphenolic compounds, flavonoids Oxygen free radical quenching, inhibition of lipid peroxidation while Alkaloids, terpenoids, are anti- oxidants, neuropharmacological agents, inhibitors of micro-organisms and reduce the risk of fungal infection (Saxena *et al.*, 2016).

Angelica genus belongs to the family Umbelliferae, only two species (*Angelica glauca*, *A. archangelica* var. *himalaica*) have been reported from the inner Himalayan ranges, Pakistan. In Pakistan both the species grow wild and have a medicinal importance (Nasir, 1972). Root and seeds of the *Angelica* species are used in traditional system of medicine, food and medicinal industry (Wiersema & Leon, 1999). It is useful against stomach diseases, scurvy, infections, cough, cold and poultice is applied in case of body ache (Fjellstrom, 1986). It has a cytoprotective and good effect on the liver which indirectly protects the liver from oxidative stress due to the excessive use of alcohol impact (Yeh *et al.*, 2003). It is also used as a flavouring for confectionary and liqueurs because of the special scent (Grieve, 1979) wines and perfumes (Duke, 1987). European legislation believes on herbal medicinal products which are authenticated as ordinary medicines. However, the approval arises of well-established herbal medicines or traditional herbal medicines from indication of use and absoluteness documentation (Madsen, 2008).

The aim of this study was to analyze the seeds of *Angelica* species physicochemically and phytochemically this study will be helpful for formulating the phytochemical standard. Thus, the preliminary information about phytochemical constituents will be useful in finding out the drug efficacy.

Materials and Methods

Seeds collection and plant identification: Seeds of *Angelica glauca* and *A. archangelica* var. *himalaica* were collected from Lawat area, Neelum valley Azad Kashmir and plant specimens were identified with the help of available literature ''Flora of Pakistan'' (Nasir & Ali, 1971-1994; Ali & Qaiser, 1995-to date). Seeds were cleaned and shade dried then ground to fine powder. Voucher specimens were deposited in (KUH) Karachi University Herbarium, Centre for Plant Conservation, University of Karachi.

Physicochemical investigation: Ash values, Water Soluble Ash value, Acid insoluble Ash value, extractive values in different solvents were investigated (Chetankumar *et al*., 2013).

Phytochemical screening: For phytochemical assessment standard methods were applied. Such as Alkaloids by Mayer's test (Evans,1997), Hager's test (Wagner *et al.*, 1996) and wagner's test (Wagner, 1993) Carbohydrate by benedict's test and Fehling's reagent (Ramakrishnan *et al.*, 1994), Protein and Amino acid by Biuret test (Gahan, 1984) and Millon's test (Resch & Swift, 1960). Phenolic compound by Lead acetate test and Ferric chloride test (Mace, 1963). Fixed oil was assessed by spot test and Saponin by Foam test, Glycoside by Boorntrager's test (Evans, 1997).

Results

Seed morphology of *Angelica glauca* Edgew.

Seeds were 12x6 mm, brown, elongated, elliptic, lateral ridges broadly winged, ribbed surface; hilum 0.5mm, basal, circular in outline (Fig. 1A, Table 1).

Seed morphology of *Angelica archangelica* var. ***himalaica*** (Clarke) E. Nasir.

Seeds were 9x4 mm, brown, oblong to sub-quadrate, dorsal ridges laterally winged, ribbed surface; hilum 0.5mm, basal, circular in outline (Fig. 1B, Table 1).

Physicochemical analysis: Physicochemical analysis of the seeds of *Angelica glauca* and *A. archangelica* var. *himalaica*, revealed the total ash value as 31% and 33%, water soluble ash was 50% and 51%, acid insoluble as 80% and 82%. Moisture content and extracting values in different solvent are given in Table 2.

Phytochemical screening of seeds of *Angelica glauca* and *A. archangelica* var. *himalaica*

Phytochemical analysis of seed extracts of *Angelica glauca* and *Angelica archangelica* var. *himalaica*, showed the presence of alkaloid, carbohydrates, protein, phenolic compounds, flavonoids, glycoside, terpenoids and fixed oil in all solvent extracts (Table 3).

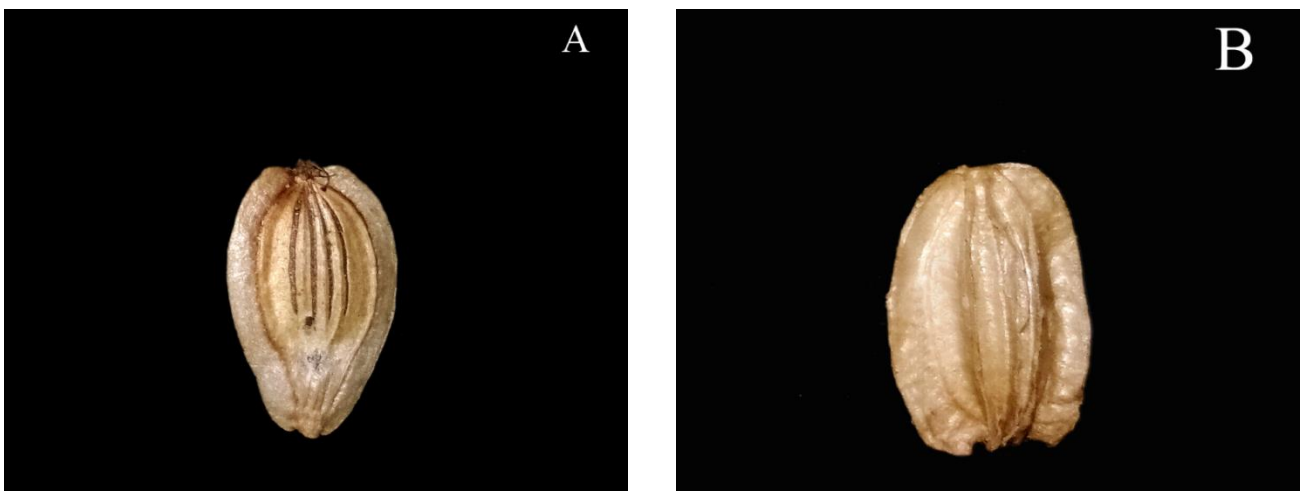

Fig. 1. Scanning of micrograph of *Angelica* seed.
A= Seed of *Angelica glauca;* B= Seed of *Angelica archaangelica* var *himalaica*

Table 1. Seed morphology of *Angelica* species.

Morphology	*Angelica glauca* Edgew.	*Angelica archangelica* var. *himalaica* (Clarke) E. Nasir
Shape	Elongated	Oblong to sub-quadrate
Length	8-12 mm	6-9 mm
Breadth	5-6 mm	3-4 mm
Ridges	lateral ridges broadly winged	Dorsal ridges laterally winged
Hilum	0.5mm	0.5mm
Color	Brown	Brown
Surface	Ribbed	Ribbed

Table 2. Physicochemical Analysis of seeds of *Angelica* species.

S. No.	Physico-chemical parameters	*Angelica glauca*	*Angelica archangelica* var. *himalaica*
1.	Physical state of ash	Fine powder	Fine powder
2.	Colour of ash	Grayish white	Grayish white
3.	% of loss on drying	8%	10%
4.	% of ash content	31%	33%
5.	Water soluble ash	50%	51%
6.	Water insoluble ash	50%	51%
7.	Acid soluble ash	20%	18%
8.	Acid insoluble ash	80%	82%
9.	Acetone soluble extractive value	1%	6.4%
10.	Chloroform soluble extractive value	1%	4.6%
11.	Methanol soluble extractive value	4%	10.4%
12.	Water soluble extractive value	10%	13%

Table 3. Phytochemical screening of seeds of genus *Angelica* L. species.

S.No.	Phytochemical tests	*Angelica glauca*				*Angelica archangelica* var. *himalaica*			
		Ac	Chl	Met	W	Ac	Chl	Met	W
1.	**Alkaloids**								
	Wagner's reagent	+	+	+	+	+	+	+	+
	Mayer's reagent	+	+	+	+	+	+	+	+
	Hager's test	+	+	++	++	+	+	++	++
2.	**Carbohydrates**								
	Benedict's test	++	++	++	++	++	++	++	++
	Fehling test	++	++	++	++	++	++	++	++
3.	**Protein and amino acids**								
	Biuret test	++	++	++	++	++	++	++	++
	Millions test	++	++	++	++	++	++	++	++
4.	**Phenolic compounds**								
	Lead acetate	+	+	++	++	+	+	++	++
	Ferric chloride test	+	+	++	++	+	+	++	++
5.	**Flavonoids**	+	+	+	+	+	+	+	+
6.	**Glycoside**								
	Salkowsk's test	++	+++	++	_	+++	++	++	++
	Keller-kilani test	+	+	++	++	++	++	++	++
7.	**Terpenoids**	+++	+++	+++	++	+++	+++	+++	++
8.	**Saponins**								
	Foam test	_	_	_	++	_	_	_	++
9.	**Fixed oil**								
	Spot test	++				++			

Key: Ac= Acetone, Chl= Chloroform, Met= Methanol, W= Water, + = Present, - = Absent, ++ = Present in moderate amount, +++ = present in high amount

Discussions

Different *Angelica* species are being used for curing in several diseases in many countries. Essential oils have been reported to cure fever, anemia, malaria, arthritis, gynecological disease and other health problem because essential oil are of low molecular weight and complex mixture of oxygenated compounds and terpenoids (Sowndhararajan *et al*, 2017). Thereby medicinal value of *Angelica* is very high. The entire genus is confined to cold regions where temperature reaches 5 to 19^{o}C (Hornok, 1992; Bomme, 2001). In Pakistan both the species *A. glauca* and *A. archangelica* var. *himalaica* are also confined to high altitudes. *Angelica* is not sold in retails market. It is only available in special firms (Bomme, 1997, 2001). The best time of seeds harvesting takes place during July and August, when seeds looks yellow or brown in color. (Galambosi, 1994). Main umbel cuts provide largest size seeds with maximum quantity (Galambosi, 1994; Dachler & Pelzmann, 1999). *Angelica* species are widely used in traditional system of medicine facing serious threats of extinction, but these species can be propagated through seeds and underground stem as well. According to IUCN red list data (Anon., 2014) *Angelica glauca* under threat due to loss of habitat, indiscriminate collection for medicine and biotic pressure. Agricultural invasion, construction of roads and human territory are the other factors responsible for population decline.

In this study majority of phytochemicals (alkaloide, carbohydrate, Fixed oil, Glycoside, Flavonoids, Terpenoids, Saponins, Phenolic compounds, Protein and amino acids) were detected in the seeds of both species in various solvent (Acetone, chloroform, methanol, water) extracts. These phytochemicals are reported to have various therapeutic and biological activities. In both species terpenoids were found in high quantity while, others compounds like carbohydrate, protein and amino acid, phenolic compounds, glycoside except alkaloids, saponins, fixed oil were found in moderate quantity. Saponin was only detected in water extract but it was absent in other extracts. For alkaloids best result was achieved by Hager's reagent. While, Million's reagent was best for the amino acid and protein screening. Similarly Salkowsk's test and Keller-kilani test gave better result for glycosides. Various bioactive compounds were detected through this study; so, on the basis of current study seeds of *A. glauca* and *A. archangelica* var *himalaica* can be counted in those species which are rich in terms of secondary metabolites Medicinal value of such species is very high. There is no major difference of chemicals between the two species. Phytochemical results are mentioned in a table (3).

Seeds of *Angelica* species were found as a rich source of bioactive compounds. Best extraction was done in water and methanol in both species, in *A. glauca* extractive value was 4% and 10% where as in *A. archangelica* var *himalica* it was 10.4% and 13% by mass. Water was best solvent followed by methanol while, extractive values in acetone and chloroform were very low. Similarly ash solubility of both species was high in water that is 50% and 51% and low in acid which is 20% and 18%. The amount of phenolic compounds in acetone and chloroform extracts in both species was low whereas, it was moderate in methanol and water extracts. Our study showed that the seeds of *A. glauca* and *A. archangelica* var *himalaica* had number of phytochemicals classes. Richness of phytochemicals in any plant sources makes it medicinally important.

Acknowledgment

We are grateful to Higher Education Commission for providing grant for this research under Project 20-3847/NRUP/R&D/HEC/14.

References

Anonymous. 2014. The IUCN Red List of Threatened Species, Version 3.1. [downloaded on 2019 August 29]. http://www.iucnredlist.org

Bomme, U. 1997. Neue Herkünfte bei Engelwurz und Baldrian verfugbar. Gemuse 11/1997. 637-638.

Bomme, U. 2001. Kulturanleitung für Engelwurz. Edn 4 . Bayerische Landesanstalt für Land-wirtschaft (LfL), Freising, Deutschland.

Charbonneau, J., M.H. Michaud, A.Gosselin, C. Martel and N. Tremblay. 1993. Effect of substrate and soil type on angelica root productivity. *Acta Hort*., 331-338.

Chetankumar, N. and P.N. Patel. 2013. Physicochemical and Phytochemical evaluation of *Dendrobium acraie* roots. *Int. J. Res. Pharm. & Biomed. Sci.*, 4(1): 75-80.

Dachler, M. and H. Pelzmann. 1999. Engelwurz. In: *Arznei - und Gewürzpflanzen: Anbau, Ernte, Aufbereitung*. 2nd Edition. Österreichischer Agrarverlag, Klosterneuburg, Austria. pp. 169-171.

Duke, J. 1987. Handbook of Medicinal Herbs. *Boca Raton*, USA: CRC Press Inc.

Evans, W.C. 1997. Trease and Evans Pharmacognosy. 14thEdn. Harcourt Brace and company. Asia Pvt. Ltd Singapore.

Fatima, H, A. Perveen, S. Qamarnnisa and U. Munir. 2014. Pharmacognostic and phytochemical analyses of leaves and seeds storage of *Abutilon pakistanicum*. An Endemic plant of Pakistan. *Pak. J. Bot*., 46(6): 2035-2041.

Fjellström, P. 1986. Samisk Folkmedicin. In: Samernas Samhälle I tradition och nutid. 2nd Edition. Nordstedts Förlag, Sweden, pp. 353-355.

Galambosi, B. 1994. Okologisk Urtedyrkningi Norden. NHL−Fagtjenesten. As, Norway. 95-97.

Ghan, P.B. 1984. Plant Histochemistry and cytochemistry: An Introduction . Academic press, Florida, U.S.A.

Grieve, M. 1979. A Modern Herbal. Jonathan Cape Ltd, London. pp. 35-40.

Hornok, L. 1992. Angelica. In: *Cultivation and processing of medicinal plants*. John Wiley & Sons, Chichester, UK. pp. 147-150.

Mace, M.E. 1963. Histochemical Localization of phenols in healthy and diseased tomato roots. *Phytopathol.*, 16: 915-925.

Madsen. S. 2008. Does traditional use of herbal remedies guarantee Safety: Bioactive compounds in plants – benefits and risks for man and animals Proceedings from a symposium held at. The Norwegian Academy of Science and Letters, Oslo, 13-14.

Mahato, S.B. and S. Sen. 1997. Advances in triterpenoid research, 1990-1994. *Phytochem.*, 44: 1185-1236.

Nasir, E. 1972. Umbelliferae. In: Nasir, E. & Ali, S.I. (Eds.) *Flora of Pakistan*. 20: 1-169, Islamabad.

Newman, D.J. and G.M. Cragg. 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Natural Prod*., 75(3):311-35.

Raaman, N. 2006. Phytochemical Techniques, New India Publishing Agency Pitam Pura, New Delhi- 100088.

Ramakrishnan, S., K.G. Prasanan and R. Rajin. 1994. Text book of medicinal biochemistry. Orient Longman, New Delhi, India.

Rasch, E. and F. Swwift. 1960. Micro Photometric analysis of the cytochemical million reactions. *J. Histochem. Cytochem*., 8: 4-17.

Saxena, M., J. Saxena, R. Nema, D. Singh and A. Gupta. 2013. Phytochemistry of Medicinal Plants. *J. Pharmacog. & Phytochem*, 1(6): 168-182.

Sowndhararajan, K., P. Deepa, P. Kim, J.S. Park and S. Kim. 2017. A review of the composition of the essential oils and biological activities of angelica species. *Scientia Pharmaceutica*, 85(3): 33: doi:10.3390/scipharm85030033

Wagner, H. 1993. Pharmazeutische Biologic, Edn 5, AUFI.15 BN3-437-20 498-X. Gustav fisher Vwelag, Stuttgart, Germany.

Wagner, H., Z. Bladt and E.M. Gain. 1996. Plant drug analysis. Springer Veralag, Berlin, Germany. pp. 360.

Wiersema, J.H. and B. Leon. 1999. World economic plants: A standard reference. Florida, USA: CRC Press.

Yeh, M.L., C.F. Lui, C.L. Huang and T.C. Huang. 2003. Hepatoprotective effects of *Angelica archangelica* in chronically ethanol−trated mice. *Pharmacol.*, 68:70-73.

(Received for publication 18 July 2018)