

**Original Article****Phytochemical Screening of *Aquilegia nivalis* Flax Jackson: An Important Medicinal Plant of Kashmir Himalaya: A Perspective**Mudasar Ahmad^{1,3}, Zahoor A. Kaloo^{1,3}, Bashir A. Ganai², Ubaid Yaqoob³, Hilal A. Ganaie²¹ Plant Tissue Culture Research Laboratory, Department of Botany, University of Kashmir, Srinagar-190 006, J & K, India.² Phytochemistry Research Laboratory, Centre of Research for Development, University of Kashmir, Srinagar-190 006, J & K, India.³ Department of Botany, University of Kashmir, Srinagar-190 006, J & K, India.

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E-mail address: bbcganai@gmail.com**Running Title:** Phytochemical Screening of *Aquilegia nivalis* Flax Jackson**Received: 09 November, 2016; Revised: 27 December, 2016; Accepted: 20 January, 2017**Available online at <http://www.thescientificpub.com><http://dx.doi.org/10.19046/abp.v04i01.02>**Abstract**

Medicinal plants contain bioactive compounds which are used for the treatment various ailments. Phytochemicals have been categorized in two categories, primary and secondary metabolites. Primary include chlorophyll, proteins, sugar and amino acids. Secondary constituents include terpenoids and alkaloids. The present study involves the phytochemical screening of endemic perennial medicinal herb - *Aquilegia nivalis* which is reported from Kashmir Himalaya. The aerial parts and rhizomes of the selected medicinal plant were washed, air dried and then powdered for qualitative and quantitative analysis. Different extracts of aerial parts and rhizomes were used to find out the phytochemical constituents in the *A. nivalis*. The main objective of this work was to check the presence or absence of the phytochemical constituents and quantitative analysis of alkaloids, phenols and saponin in methanolic extracts of aerial parts and rhizomes of *A. nivalis*. The results of the phytochemical analysis of *A. nivalis* showed that the terpenoids, phlobatannins, flavonoids and alkaloids were present. The phytochemical analysis of medicinal plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing various diseases. It is expected that the important phytochemical constituents detected in *A. nivalis* found in Kashmir Himalaya may prove as important source of novel phytochemicals.

Keywords: *Aquilegia nivalis*, phytochemical screening, quantitative analysis, medicinal plants.**Introduction**

The medicinal plants are useful for healing as well as for curing human diseases because of the presence of phytochemical constituents [1]. Phytochemicals are naturally occurring in the medicinal plants as a defense mechanism and protection to various diseases. Chlorophyll, proteins and common sugars are included as primary constituents and secondary compounds include

terpenoid, alkaloids and phenolic compounds [2]. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities [3]. Terpenoids are very important in attracting useful mites that consume the herbivorous insects [4]. Alkaloids are used as anesthetic agents and are found in medicinal plants [5].

The exploitation of plants by man for the treatment of diseases has been in practice for a very long time. Herbal drug constitutes a major part in all the traditional system of medicines [6]. Screening of compounds obtained from plants for their pharmacological assay has indeed been the vast source of innumerable therapeutic agents representing molecular diversity engineered by nature. It is therefore necessary and urgent to fight against emerging and reemerging infectious diseases. Further, newer strains are being continuously discovered which are refractory to the current arsenal of drugs [7]. The World Health Organization (WHO) estimated that 80% of the population of developing countries rely on traditional medicine mostly plant drugs. Medicinal plants are being natural, non-narcotic, having no side effect. Demand for medicinal plants is increasing in both developing and developed countries. Over the past few decades; there has been much interest in natural materials as source of new antibacterial agents. Different extracts from traditional medicinal plants have been tested. Many reports show the effectiveness of traditional herbs against microorganisms. As a result, plants have become one of the bases of modern medicine [8]. Natural products of higher plants may give a new source of antibacterial agents with possibly a novel mechanism of action. The selection of crude plant extract for screening the antibacterial activity has the potential of being more successful in the initial steps than screening of pure compounds [9]. The present study involves qualitative and quantitative analysis of the endemic perennial medicinal herb - *Aquilegia nivalis* for the first time.

Materials and Methods

Collection and identification of plant material

A. nivalis Flax Jackson is an endemic perennial medicinal herb of Ranunculaceae family. It is commonly known as Zao Neil. The *A. nivalis* was collected from Apherwat area of Gulmarg. The plant was identified by the plant taxonomy division of the Department of Botany with voucher specimen No. 1821-KASH. The present study was undertaken for qualitative and quantitative analysis of the hexane, ethyl acetate, methanolic and aqueous extracts of aerial and rhizome parts *A. nivalis*.

Preparation of plant extracts and phytochemical screening

The whole plant material was washed 2-3 times with running water and once with distilled water, dried in shade for 5-8 days, grinded to fine powder and stored in airtight container at room temperature in the dark until used. 60 g of shade-dried powder was filled in the thimble and

extracted with hexane, ethyl acetate, methanol and aqueous extract for 72h. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in air tight bottle till further use.

Measured amount of the powdered plant material was successively extracted in a Soxhlet extractor at elevated temperature using n-hexane which was followed by ethyl acetate, methanol and water. All extracts were filtered individually through filter paper and poured on petridishes to evaporate the liquid solvents from the extract to get dry extracts. After drying, crude extracts were stored in stock vials and kept in refrigerator for further use. Percent of yield [10] was calculated as follows:

$$\text{Extraction Yield\%} = \frac{W_1}{W_2} \times 100$$

Where, W_1 = Net weight of powder in grams after extraction and W_2 = Total weight of powder in grams taken for extraction.

Chemicals used

Fehling solution A and Fehling solution B, ethanol, distilled water, aqueous HCl, methanol, Ethyl acetate, concentrated sulphuric acid, Ammonia solution, picric acid, Hexane, Isoamyl alcohol, Ferric chloride.

Phytochemical screening of aerial and rhizome extracts of *Aquilegia nivalis*

The aerial and rhizome extracts of *A. nivalis* were screened for the presence of major bioactive constituents like alkaloids, phenolics, flavonoids, tannins, cardiac glycosides, terpenes, anthraquinone glycosides saponins, steroids, and carbohydrates using standard qualitative phytochemical methods as described by Trease and Evans [11] and Harborne [12].

Qualitative screening

Tannins: To 2 ml of aqueous extract 2 ml of 5% $FeCl_3$ was added. Formation of yellow brown precipitate indicates that tannins are present [13].

Alkaloids: To the 2 ml Methanolic filtrate, 1.5 ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Mayors reagents/Wagner's reagent/ Dragendroff reagent was added. Formation of orange precipitate indicates the presence of alkaloids [14].

Saponins: Aqueous extract of 2 g powder was made and subjected to frothing test. Frothing persistence indicated presence of saponins. Later the froth was mixed with few drops of olive oil. Formation of emulsion indicates presence of saponins [15].

Cardiac glycosides: To 2 ml alcoholic filtrate, 1 ml glacial acetic acid and 1-2 drops of $FeCl_3$ was added followed by

1 ml of concentrated H_2SO_4 . Appearance of brown ring at the interface indicates presence of cardiac glycosides. A violet ring may also appear below the brown ring [11].

Flavonoids: 2g plant material was extracted in 10 ml alcohol or water. To 2 ml filtrate few drops of concentrated HCl followed by 0.5 g of zinc or magnesium turnings was added. After 3 minutes, magenta red or pink color indicated the presence of flavonoids [13].

Phenolics: To 2 ml of alcoholic or aqueous extract, 1 ml of 1% ferric chloride solution was added. Blue or green color indicates phenols [16].

Test for proteins: Biuret test: Added 4% of NaOH and few drops of 1% $CuSO_4$ solution to 3 ml of the extract. Formation of violet or pink colour indicates the presence of proteins [17].

Test for carbohydrates: Monosaccharide Barfoed's test: Mix equal volumes of Barfoed's reagent and the extract solution. Heated for 1–2 min in a boiling water bath and cooled. Red colour was observed [17].

Test for carbohydrates: Molisch test: To 2–3 ml of the aqueous extract, add two drops of alpha naphthol solution in alcohol, shake and add conc. H_2SO_4 from the sides of test tube. Violet ring is formed [17].

Test for steroids: The powder samples of *A. nivalis* (1 gm) were dissolved in chloroform (10 ml) and added concentrated sulphuric acid (1 ml) into the test tube by wall sides. The colour of the upper layer turned red and the sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for phlobatannins: Plant powder sample was mixed with distilled water in a test tube, then shaken it well, and filtered to take plant extract. Then to each plant extract, 1% aqueous hydrochloric acid was added and each plant sample was then boiled with the help of Hot plate stirrer. Formation of red colored precipitate confirmed a positive result.

Salkowski reaction test for phytosterols: To 0.5 mL chloroform extract in a test tube add 1 mL of Concentrated (conc.) H_2SO_4 from the sides of the test tube. Appearance of reddish brown colour in chloroform layer indicates presence of phytosterols.

Ninhydrin Test: To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Quantitative screening

Determination of total phenols by Spectrophotometric method

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15

minutes. 5ml of extract was pipetted into a 50ml flask, then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol was also added. The samples were made up to mark and left to react for 30 minutes for colour development. This was measured at 505nm.

Alkaloid determination

3g of the powder was extracted using 200 ml of 10% acetic acid in ethanol. The solution was covered for almost 4 hours. Filtrate was concentrated to 25 ml. Concentrated ammonium hydroxide was added stepwise to attain precipitation. The whole solution was kept as such so that precipitate will settle. Collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded and pellet obtained was dried and weighed [18, 19].

Tannin determination

500mg of the sample was weighted into a 50ml bottle. 50ml of distilled water was added and shaken for 1hr in a shaker. This was filtered into a 50ml volumetric flask and made up to mark. Then 5ml of the filtered was pipette out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008 M Potassium Ferro cyanide. The absorbance was measured at 120nm [20].

RESULTS

The results of preliminary phytochemical analysis are tabulated in **Table 1**. The phytochemical study revealed the presence of various phytoconstituents in all the extracts. In the hexane extract of aerial part of *A. nivalis* various phytoconstituents like alkaloids, flavonoids, cardiac glycosides, steroids, triterpenoids and phytosterols was present except phenols, tannins, saponins, proteins, amino acids and phlobatannins. Ethyl acetate showed the presence of alkaloids, phenols, tannins, carbohydrates, proteins, amino acids and phlobatannins. However, saponins, flavonoids, terpenoids cardiac glycosides, steroids and triterpenoids were absent. Where as in methanolic extract only alkaloids, phenols, tannins, cardiac glycosides, flavonoids, saponins, triterpenoids, proteins, amino acids, carbohydrates, phytosterols were found to be present, while the rest of the compounds were found to be absent. In the aqueous extract of *A. nivalis* tannins, saponins, carbohydrates, proteins, amino acids and phlobatannins were present, whereas alkaloids, cardiac glycosides, phenols, phytosterols, flavonoids, steroids, triterpenoids and phytosterols were tested absent. Hexane extract of rhizome part of *A. nivalis* showed the presence of all phytochemicals analyzed except phenols, cardiac glycosides, flavonoids, saponins, carbohydrates, proteins, amino acids and phlobatannins. However, in the

ethyl acetate extract of rhizomes tannins, phenols, cardiac glycosides, steroids, saponins, triterpenoids, proteins, amino acids, carbohydrates were present and rest of the phytochemicals were absent. In the methanolic rhizome extract of *A. nivalis* tannins, cardiac glycosides, saponins, flavonoids, triterpenoids, carbohydrates, proteins, amino acids, phytosterols and phlobatannins were present, whereas alkaloids and phenols were tested absent. In the aqueous rhizome extract phenols, tannins, saponins, alkaloids, cardiac glycosides, terpenoids, alkaloids, carbohydrates, amino acids, proteins, phytosterols and phlobatannins were present, whereas flavonoids, steroids, triterpenoids were found to be absent.

The percentage in methanolic extracts of aerial and rhizomes parts of *A. nivalis* is summarized in **Table 2**. *A. nivalis* aerial part contains the highest percentage of phenols (0.98 %) followed by tannins (0.37 %) and alkaloids (0.27 %). The rhizome extract of *A. nivalis* contains 3.26 % alkaloids, 0.38 % phenols and 0.29 % tannins. It is obvious from the results that aerial part of *A. nivalis* contains highest percentage of phenols, while as highest percentage of alkaloids were recorded in rhizome part (3.28 %).

Figure 1: Qualitative phytochemical screening of rhizome and aerial parts of extracts of *Aquilegia nivalis*

Phytoconstituents	Test	Aerial part				Rhizome			
		Hexane	Ethyl Acetate	Methanol	Aqueous	Hexane	Ethyl Acetate	Methanol	Aqueous
Alkaloids	Wagner's test	+ve	+ ve	+ ve	- ve	+ ve	- ve	- ve	+ ve
Phenolics	phenol test	-ve	++ ve	++ ve	- ve	- ve	+ ve	- ve	+ ve
Tannins	Ferric chloride test	- ve	++ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Cardiac glycosides	Keller-Killani test	+ ve	- ve	+ ve	- ve	- ve	+ ve	+ ve	+ ve
Flavonoids	Shinoda's test	+ ve	- ve	+ ve	- ve	- ve	- ve	+ ve	- ve
Saponins	Frothing test	- ve	- ve	+ ve	+ ve	- ve	+ ve	+ ve	+ ve
Steroids	Libermann-Buchard's test	+ ve	- ve	- ve	- ve	+ ve	+ ve	+ ve	- ve
Triterpenoids	Libermann-Buchard's test	+ ve	- ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve
Carbohydrates	Barfoed's test	- ve	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	+ ve
Proteins	Biuret Test	- ve	+ ve	+ ve	+ ve	-ve	+ ve	+ ve	+ ve
Amino acids	Ninhydrin Test	- ve	+ ve	+ ve	+ ve	- ve	+ ve	+ ve	+ ve
Phytosterols	Salkowski Test	+ ve	- ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve
Phlobatannins		-ve	+ ve	+ ve	+ ve	- ve	- ve	+ ve	+ ve

(++) = strong presence, (+) = moderate presence

Discussion

Plant derived substances have recently gained great interest owing to their versatile applications. Medicinal plants are the richest bio resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [21]. All plants produce chemical compounds as part of their normal metabolic activities. These include primary metabolites found in smaller range of plants, some useful ones found only in a particular genus or species [22]. Herbalists tend to use extracts from parts of plants, such as the roots or leaves but didn't isolate particular phytochemicals. Pharmaceutical medicine prefers single ingredients on the grounds that dosage can be easily quantified [23]. Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, bio synthetic origin and functional groups into primary and secondary metabolites [24].

Successive isolation of compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but in the present investigations the ethanolic extract provided more phyto-compounds followed by acetone in comparison to the aqueous extraction which are in agreement with previous researchers [25, 26]. The qualitative changes in the phytochemical analysis of tested plant species are correlated to the methods of preparation. The preliminary phytochemical tests are therefore significant and helpful in finding chemical constituents in the plant material [27, 28] that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds [24]. The preliminary phytochemical studies during the present investigations revealed that the *A. nivalis* is mainly constituted of various primary and secondary metabolites which can be quantified for

application in pharmaceutical industry, while other plant species also showed promising results, which can also be quantified.

Conclusions

The preliminary phytochemical analysis revealed that these phyto-compounds are mainly present in the methanolic extract as compared to hexane, ethyl acetate and aqueous extract of both aerial and rhizome extract of *A. nivalis*. The present study will prove useful in the comparative studies of the amount of bioactive principles present in this herb with its other species and populations belonging to different regions with different climatic conditions. This data can also help us to choose the superior race of this valuable herb with greater quantity of medically and therapeutically important phytochemicals. It may be concluded that *A. nivalis* may prove as important source of novel Phytochemical.

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Conflict of interest

There is no conflict of interest to reveal.

Table-2: Percentage of alkaloids, phenol and tannins in methanolic extract of aerial and rhizome parts of *Aquilegia nivalis*

	Alkaloids	Phenol	Tannins
<i>Aquilegia nivalis</i> (aerial)	0.27 ± 0.10	0.98 ± 0.11	0.37 ± 0.10
<i>Aquilegia nivalis</i> (rhizome)	3.26 ± 0.12	0.38 ± 0.19	0.29 ± 0.19

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