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Phytochemical Screening of *Meconopsis aculeata* Royle an Important Medicinal Plant of Kashmir Himalaya: A Perspective

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ABSTRACT

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities. The present study involves medicinal plant *Meconopsis aculeata* which is locally available in Kashmir Himalaya. The aerial and rhizome parts of the selected medicinal plant were washed, air dried and then powdered for qualitative and quantitative analysis. Different extracts of aerial and rhizome parts were used to find out the phytochemical constituents in the *Meconopsis aculeata*. The main objective of the research work was to check the presence or absence of the phytochemical constituents and quantitative analysis in different extracts of *Meconopsis aculeata*. The results of the phytochemical analysis of *Meconopsis aculeata* 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 of various diseases. It is expected that the important phytochemical constituent recognized in *Meconopsis aculeata* found in Kashmir Himalaya will be very useful in the curing of various diseases of this region.

Key words: *Meconopsis*, phytochemical, quantitative, analysis

INTRODUCTION

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (Choudhary *et al.*, 2013; Nostro *et al.*, 2000; Rao and Savithramma, 2012). Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (Krishnaiah *et al.*, 2007). Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities (Mahato and Sen, 1997). Terpenoids

are very important in attracting useful mites and consume the herbivorous insects (Kappers *et al.*, 2005). Alkaloids are used as anesthetic agents and are found in medicinal plants (Herourat *et al.*, 1988; Kumbhar and Godghate, 2015).

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 (Higa *et al.*, 1994). 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 (Erturk *et al.*, 2006). The World Health Organization (WHO) estimated that 80% of the population of developing countries rely on traditional medicine mostly plant drugs, for their primary health care needs. 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 (Evans *et al.*, 2002; Chothani and Patel, 2012). Natural product 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 (Chandrappa *et al.*, 2010). As no such work has been done on the phytochemical screening of the plant, so the present study was carried out to explore the phytoconstituents of the plant.

MATERIALS AND METHODS

Collection of plant material: *Meconopsis aculeata* is an endemic perennial medicinal herb of Papaveraceae family (Fig. 1). It is commonly known as achatsarmum. The plant bears a single, unbranched, erect, hard and prickly stem. Single raceme bears many flowers. Flower is showy,



Fig. 1: Mother plant

actinomorphic, hermaphrodite, complete and hypogynous. Flower has a thin, cylindrical, bristly and erect pedicel. Petals are four, obtuse, obovate, delicate, thin, soft and with wavy margins. Fruit is a many seeded capsule.

The whole plant of *Meconopsis aculeata* was collected from Sinthan top area of Anantnag district. The plant was identified by the plant taxonomy division of the Department of Botany with voucher No: JKASH/CBT/15 Dated 25. 02. 2015. The present study was undertaken for qualitative and quantitative analysis of the hexane, ethyl acetate, methanolic and aqueous extracts of *Meconopsis aculeata*. *Meconopsis* is a genus of flowering plants in the family Papaveraceae. It was first described by French botanist Viguier in 1814, who named it as poppy-like (gr. mekon poppy oopsis alike). The other 40 species are found in the Himalayas. *Meconopsis aculeata* is a blue flowered thorny species of the genus *Meconopsis* with a small geographical distribution restricted to specific areas of Kashmir Himalaya at an altitude of 3000-4000 m. The species is highly valued as a medicinal plant and the resulting demand for the plant as medicine has placed pressure on wild populations due to over-collection. The *Meconopsis* species occurs as small populations among rocks or in rock crevices on very steep, least stable, moist shady or open slopes.

Extraction of plant material: The whole plant material was washed with 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 for 72 h. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in air tight bottle till further use (Fig. 2).

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 aqueous. 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. Percentage of Yield (Patil and Gaikwad, 2010) was calculated as follows:

$$\text{Extraction yield (\%)} = \frac{W_1}{W_2} \times 100$$

where, W_1 is net weight of powder in grams after extraction and W_2 is total weight of powder in grams taken for extraction.

Chemicals: Fehling solution A and Fehling solution B, ethanol, distill 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 *Meconopsis aculeata*: The aerial and rhizome parts of *Meconopsis aculeata* 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 (1989) and Harborne (1973).

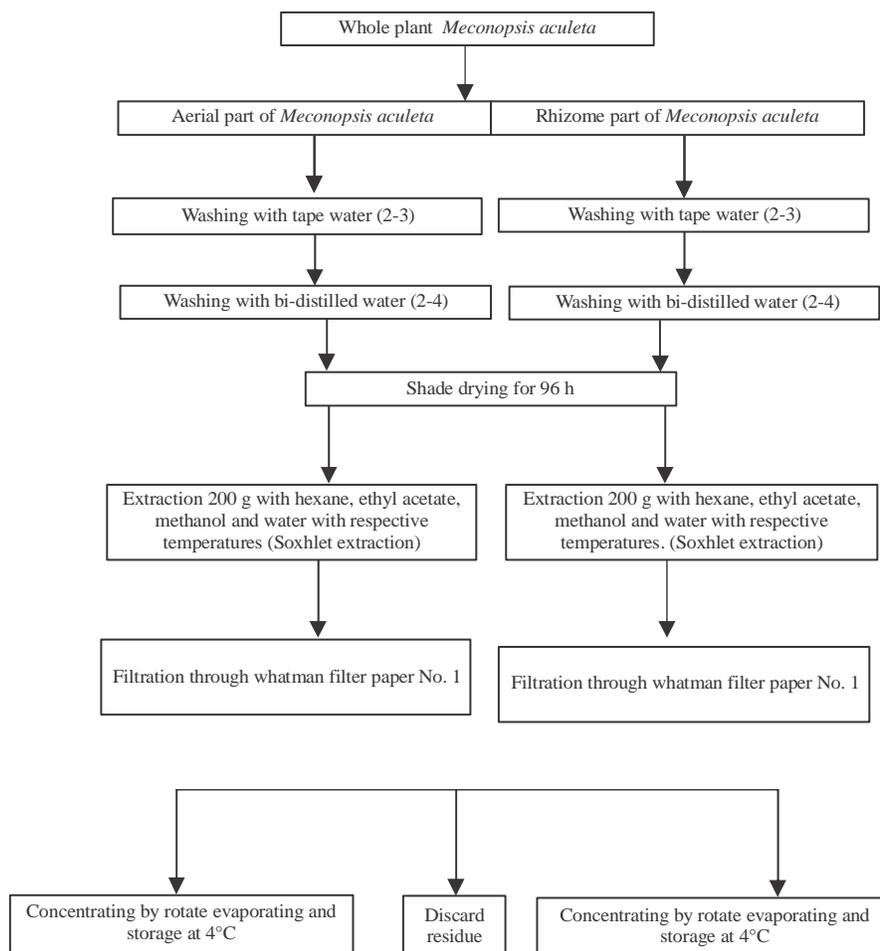


Fig. 2: Procedure used in the extraction

Qualitative screening

Tannins: To 2 mL of aqueous extract 2 mL of 5% FeCl₃ was added. Formation of yellow brown precipitate indicates that tannins are present (Parekh and Chanda, 2007).

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 (Oguyemi, 1979).

Saponins: Aqueous extract of 2 g powder was made and subjected to frothing test. Frothing persistence indicated presence of saponins. Latter the froth was mixed with few drops of olive oil. Formation of emulsion indicates presence of saponins (Sofowora, 1993).

Cardiac glycosides: To 2 mL alcoholic filtrate, 1 mL glacial acetic acid and 1-2 drops of FeCl₃ was added followed by 1 mL of concentrated H₂SO₄. Appearance of brown ring at the interface indicates presence of cardiac glycosides. A violet ring may also appear below the brown ring (Trease and Evans, 1989).

Flavonoids: About 2 g 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 min magenta red or pink color indicated the presence of flavonoids (Parekh and Chanda, 2007).

Phenolics: To 2 mL of alcoholic or aqueous extract, 1 mL of 1% ferric chloride solution was added. Blue or green color indicates phenols (Martinez and Valencia, 2003).

Test for proteins

Biuret test: Added 4% of NaOH and few drops of 1% CuSO₄ solution to 3 mL of the extract. Formation of violet or pink colour indicates the presence of proteins (Boxi *et al.*, 2010).

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 (Boxi *et al.*, 2010).

Test for reducing sugars

Fehling test: Mixed 1 mL of Fehling's A and Fehling's B solutions, boiled for 1 min. Added equal volume of test solution. Heated in a boiling water bath for 5-10 min. First a yellow then a brick red ppt. was observed (Boxi *et al.*, 2010).

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. H₂SO₄ from the sides of test tube. Violet ring is formed (Boxi *et al.*, 2010).

Test for steroids: The powder samples of *Meconopsis aculeata* (1 g) 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 distill 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: About 0.5 mL chloroform extract in a test tube add 1 mL of concentrated (conc.) H₂SO₄ 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 50 mL of ether for the extraction of the phenolic component for 15 min. About 5 mL of

Table 1: Percentage of crude alkaloids, phenol and tannins in aerial and rhizome part of *Meconopsis aculeata*

Plants	Alkaloids	Phenol	Tannin
<i>Meconopsis aculeata</i> (Aerial)	0.28±0.12	10.2±0.12	0.40±0.11
<i>Meconopsis aculeata</i> (Rhizome)	4.30±0.10	0.46±0.20	0.15±0.20

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

Alkaloid determination: About 3 g of the powder was extracted using 200 mL of 10% acetic acid in ethanol. The solution was covered for almost 4 h. 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 (Edeoga *et al.*, 2005; Okwu and Josiah, 2006).

Tannin determination by Van Buren and Robinson's method: About 500 mg of the sample was weighted into a 50 mL bottle. About 50 mL of distilled water was added and shaken for 1 h in a shaker. This was filtered into a 50 mL volumetric flask and made up to mark. Then 5 mL of the filtered was pipette out into a test tube and mixed with 2 mL of 0.1 M FeCl₃ in 0.1 N HCl and 0.008 M potassium ferro cyanide. The absorbance was measured at 120 nm. The percentage of crude alkaloids, phenols and tannins are given in Table 1 (Van Buren and Robinson, 1969).

RESULTS AND DISCUSSION

Plant derived substances have recently become of 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 (Choudhary *et al.*, 2013; Ncube *et al.*, 2008).

The results of preliminary phytochemical analysis are tabulated in Table 2. The phytochemical study revealed the presence of various phytoconstituents in all the extracts. In the hexane solvent extract various phytoconstituents like, flavonoids, cardiac glycosides, amino acids, carbohydrates phytosterols terpenoids and steroids were present in *Meconopsis aculeata* except phenols, saponins proteins, triterpenoids and tannins. However, ethyl acetate solvent extracts, saponins, flavonoids, terpenoids cardiac glycosides were absent and other compounds were found to be present. Where as in methanolic extract only phenols, saponins, terpenoid, cardiac glycosides proteins, amino acids, carbohydrates phytosterols and tannins were found to be present, while the rest of the compounds were found to be absent (Table 1).

In the aqueous solvent extract of *Meconopsis aculeata* tannins, saponins, alkaloids, triterpenoids terpenoids and proteins were present, whereas cardiac glycosides, phenols, amino acids, carbohydrates, phytosterols, flavonoids, steroids were tested absent.

Methanolic extract of rhizome part of *Meconopsis aculeata* showed the presence of all phytocompounds analyzed except phenols, triterpenoid, alkaloids, amino acids, carbohydrates and triterpenoids. However, in the rhizome aqueous extract tannins, phenols, saponins, terpenoids, proteins, alkaloids, amino acids, carbohydrates, triterpenoids were present rest of the phytocompounds were absent. In the rhizome hexane extract, tannins, saponins, steroids,

Table 2: Qualitative phytochemical screening of rhizome and aerial parts of extracts of *Meconopsis aculeta*

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	Liebermann-Buchard's test	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve
Triterpenoids	Liebermann-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

terpenoids, alkaloids, triterpenes and phytosterols were present, whereas phenols, flavonoids, cardiac glycosides and proteins, amino acids were found to be absent.

Ethyl acetate rhizome extract of *Meconopsis aculeta* showed the presence of phytochemicals like tannins, phenols, saponins, steroids, cardiac glycosides, proteins, amino acids, carbohydrates and phytosterols other compounds such as flavonoids, terpenoids, alkaloids and triterpenes were found to be absent.

Quantitative estimation of the percentage in *Meconopsis aculeta* is summarized in Table 2. *Meconopsis aculeta* aerial part contains the highest percentage of phenols (10.2%) followed by alkaloids (0.28%) and tannins which occurs at a percentage of (0.40%). In rhizome extract of *Meconopsis aculeta* contains alkaloids, (4.30%) phenols (0.46%) and tannins (0.15%). It is obvious from the results that aerial part of *Meconopsis aculeta* contains highest percentage of phenols, while as highest percentage of alkaloids were recorded in rhizome part.

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 (Stepp, 2004). Herbalists tend to use extracts from parts of plants, such as the roots or leaves but not isolate particular phytochemicals. Pharmaceutical medicine prefers single ingredients on the grounds that dosage can be easily quantified (Vickers, 2007). 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 (Sharanabasappa *et al.*, 2007).

Successive isolation of botanical 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 plant extract by ethanol provided more phytochemicals followed by Acetone in comparison to the aqueous extraction which are in agreement with previous researchers (Romero *et al.*, 2005; Pandith, 2009; Chothani and Patel, 2012). The qualitative changes in the phytochemical analysis of tested plant species are correlated to methods of preparation. The preliminary phytochemical tests are therefore significant and helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds (Sharanabasappa *et al.*, 2007; Kumbhar and Godghate, 2015).

The preliminary phytochemical studies during the present investigations revealed that the genus *Meconopsis aculeta* 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.

CONCLUSION

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 *Meconopsis aculeta* may be used to cure some common and other various diseases.

Hence, the preliminary phytochemical analysis revealed that these phytochemicals are mainly present in the methanolic extract as compared to hexane, ethyl acetate and aqueous extract of both aerial and rhizome extract of *Meconopsis aculeta*. So, the methanolic extract of the samples of plant material were found to contain the required major phytochemicals and other nutritive compounds needed by the pharmaceutical companies as well as in food supplements. The quantitative analysis of these phytochemicals will be an interesting area for further study.

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