Int J Pharm Bio Sci 2015 April; 6(2): (P) 194 - 200

Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PHYTOCHEMICAL SCREENING AND TLC PROFILING OF DIFFERENT EXTRACTS OF LEAVES, ROOTS AND STEM OF *ACONITUM HETEROPHYLLUM* A RARE MEDICINAL PLANT OF HIMALAYAN REGION

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ABSTRACT

Aconitum heterophyllum plants were studied for the presence of phytochemicals. Methanol extracts of leaves, roots and stems show the presence of corresponding phytochemicals like alkaloids, carbohydrates, protein, amino acid, cardiac glycosides, phenols, flavonoids, saponins, terpenoids and quinones. However the leaves, roots and stem methanol extracts do not shows the presence of Glycosides.TLC profiling of all plant extracts also give an idea about the presence of these phytochemicals. Rf (Retention factor) value of different phytochemicals provide valuable clue regarding their polarity and selection of solvents for separation of phytochemicals.

KEYWORDS: ***Aconitum heterophyllum*, phytochemical screening, TLC Profiling, & Retention factor** (Rf.).

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INTRODUCTION

Aconitum heterophyllum Wall commonly known as Atis belonging to family Ranunculaceae is a perennial herb distributed over alpine pastures of Central Himalayas, ranging altitude, 3500m to 4000m[1]. Studies on traditional medicine system have shown that the plant is used in curing hysteria, throat infection, dyspepsia, abdominal pain, diabetes and is considered as a valuable febrifuge nervine tonic especially combating debility after malaria and in hemoplageia[2]. The plant has shown to contain alkaloids heteratisine, heterophyllisine, heterophylline, heterophyllidine, atidine, isoatisine hetidine, hetsinone, benzoylheteratisine[3]. Aconitum has also shown to exhibit antipyretic, analgesic,anti-fungal, anti-bacterial,insecticidal, brime shrimp cytotoxic activities and is used to treat diseases of nervous system, digestive system, rheumatism and fever[4]. Reports have also shown that the plant possesses a good anti viral, antidiarrheal and immunostimulant properties[5]. The alkaloids mesaconitine and 3 acetylaconitine have shown to posses' anti-inflammatory activity[6[]]. In the present work phytochemical screening and TLC profiling have been attempted to root, stem, leaf extracts of *Aconitum heterophyllum*

MATERIALS AND METHODS

(1) Sample collection

Plants of *Aconitum heterophyllum* were collected from Deoban, Chakrata (District Dehradun Uttarakhand) of Garhwal Himalayas during the month of September-2013.

(2) Preparation of the extract

The collected roots, leaves and stems of *Aconitum heterophyllum* were dried in shade at room temperature and milled to a coarse powder. The obtained dried powder was subjected to continuous extraction with 85% methanol in a Soxhlet apparatus. The powdered plant material was packed in a tumble made of Whatmann's filter paper. It was extracted with methanol for 20 cycles. The extract thus obtained was concentrated in a flask evaporator under reduced pressure and controlled temperature. The yield of Methanolic extract of roots, leaves and stem of *A. heterophyllum* was 22%. The obtained residue was green and brown in colour, thick and sticky paste. The extract was stored in the refrigerator.

(3) Phytochemical Screening of Aconitum heterophyllum Extract

a. Test for the presence of alkaloids
- Mayer's test- Test solution with Mayer's reagent gives cream coloured precipitate denoting the presence of alkaloids; if alkaloids are present[7].
- Wagner's test- Test solution with Wagner's reagent gives brown coloured precipitate in presence of alkaloids[7].

b. Test for the presence of carbohydrates
- Fehling's test- Equal volume of Fehling's solution A and B were added to make the test solution. The mixture gives yellow to red precipitate if carbohydrates are present[8].

c. Test for the presence of Proteins
- Ninhydrin test- Test solution treated with a few drops of Ninhydrin reagent gives blue coloured ring on the upper layer of the sample, if proteins are present[8].

d. Test for the presence of saponin
- Foam test- The test solution was dissolved in distilled water and shaken for at least 15 minutes. The formation of foam layer indicates the presence of Saponins in the sample[9].

e. Test for the presence of phenolic compounds and tannins
- Ferric chloride test- The test solution was treated with 5% of diluted ferric chloride solution. Appearance of intense green colour shows the presence of phenolic compounds and tannins[10].

f. Test for the presence of steroids
- Salkowask'y test- Extracts were treated with few drops of conc. Sulphuric acid, shaken and allowed to stand. Appearance

of golden yellow colour indicates the presence of tri-terpenes.

g. Test for the presence of Quinones Treatment of sample with conc. Sulphuric acid gives red colour which shows the presence of quinones[11].

h. Test for the presence of Flavonoids

- Treatment of sample with dilute sodium hydroxide (NaOH) gives an intense yellow colour which becomes colourless on addition of few drops of dilute Sulphuric Acid. This indicates the presence of flavonoids.

i. Test for the presence of Terpenoids

- Treatment of sample with chloroform & concentrated sulphuric acid (2:3) gives rise to A reddish brown precipitate layer formation at the interface, indicating the presence of terpenoids[9].

j. Test for the presence of glycosides

- Bromine water test -Test solution when treated with bromine water gives yellow precipitate[11].
- Cardiac glycoside- Treatment of sample with glacial acetic acid, ferric chloride and conc. sulphuric acid gives Brown colour, indicating the presence of glycosides[11].

(4) Chromatographic Purification

TLC was carried out to isolate the principle components those were present in most effective extracts of plant. TLC studies were carried out for different extracts on Silica gel (G) 60 F. The different solvent systems of different polarities were prepared and TLC studies were carried out to select the solvent system capable of showing better resolution[12].

a. Solvent phase

The different solvent systems used were: Chloroform: Methanol (90:10), Chloroform: Methanol (60:40), Chloroform: Methanol: Ethyl acetate (40: 25: 35)

b. Method

The above prepared plant extracts were applied to pre-coated TLC plates by using capillary tubes and developed in a TLC chamber using suitable mobile phase. The developed TLC plates were air dried and observed under ultra violet light UV at both 254 nm and 366 nm. They were later sprayed with spraying reagents(10 % H_2SO_4) and some were placed in hot air oven for 1 min for the development of color in separated bands. The movement of the analyte was expressed by its retention factor (Rf). Values were calculated for different sample[12].

$$Rf = \frac{Distance\ travelled\ by\ the\ solute}{Distance\ travelled\ by\ the\ solvent\ front\ TLC\ plates}$$

c. Analytical detection

After drying the plates, they were exposed to Iodine vapors by placing in a chamber that was saturated with iodine vapors and also exposed to different spraying reagents. All plates were visualized directly after drying and with the help of UV at 254 nm and 366 nm in UV TLC viewer. The Rf value of the different spots that were observed was calculated.

RESULTS

(1) Phytochemical Analysis

The extracts of three different samples of *Aconitum heterophyllum* (Leaf, Root, and Stem) were tested for their phytochemical contents (Table-1).

Table1
Qualitative phytochemical screening of Aconitum heterophyllum extract (+Positive, - Negative)

S. No.	Chemical Constituent	Phytochemical test	*Aconitum heterophyllum* Methanolic extract		
			Leaf	Root	Stem
1	Alkaloids	Mayer's test	+	+	+
		Wagner's test	+	+	+
2	Carbohydrates	Fehling, test	+	+	+
3	Protein & Amino acid	Ninhydrin test	+	+	+
4	Saponins	Foam test	+	+	+
5	Phenolic compounds and tannins	Ferric chloride test	+	+	-
6	Steroids	Salkowask'y test	-	+	+
7	Glycosides	Bromine water test	-	-	-
		Cardiac Glycosides	+	+	+
8	Quinones		-	+	+
9	Flavonoids		+	+	+
10	Terpenoids		-	+	+

a. Methanolic extract Aconitum heterophyllum Leaves

The phytochemical analysis of the *Aconitum heterophyllum* leaf methanolic extracts showed the presence of alkaloids, carbohydrates, protein & amino acid, saponins, phenolic compounds and tannins, cardiac glycosides, quinones. Whereas, flavonoids, steroids, glycosides, and terpenoids were found absent in *Aconitum heterophyllum* leaves methanolic extract.

b. Methanolic extract Aconitum heterophyllum Roots

The phytochemical analysis of the *Aconitum heterophyllum* root extracts showed the presence of alkaloids, carbohydrates, protein & amino acid, saponins, phenolic compounds and tannins, cardiac glycosides, quinones, flavonoids, steroids, terpenoids. Glycosides were not present in *Aconitum heterophyllum* roots methanolic extract.

c. Methanolic extract Aconitum heterophyllum stem

The phytochemical analysis of the *Aconitum heterophyllum* stem extracts showed the presence of alkaloids, carbohydrates, protein & amino acid, saponins, flavonoids, steroids, cardiac glycosides, terpenoids quinones in methanol extract. Absence of phenolic compounds and tannins, glycosides was observed in the methanol extract of *Aconitum heterophyllum* stem.

(2) Thin Layer Chromatography Analysis

The retention factors (Rf) for each of the three extracts (Leaves, roots, stem) in different solvent systems are detailed below in Table 2.

Table 2
TLC of different extract of Aconitum heterophyllum

Sl. No.	Extract	Mobile Phase	No. of spots	Rf value
1	Methanol Leaves extract	$CHCl_3$:CH_3OH 90:10	9	0.097,0.125,0.208,0.278,0.388,0.625,.694,0.763 0.833
2	Methanol Stem extract	$CHCl_3$:CH_3OH 60:40	7	0.074,0.194,0.283, 0.388,0.477,0.582, 0.850
3	Methanol Root extract	CH_3OH:$CHCl_3$:EA 40:25:35	7	0.206,0.330,0.507, 0.6340.746,0.841, 0.920

a. Methanolic extract Aconitum heterophyllum Leaves

TLC analysis also suggests the presence of different kinds of phytochemicals in leaves extract. Table 2 reports the Rf values for various extracts and Figure 1 (A) shows photographs of the studied TLC slides. TLC of plant extract in Chloroform and Methanol reports nine spots for various phytochemicals. The reported spots are separated with enough space and having various Rf values showing the presence of atleast nine phytochemicals in Chloroform and Methanol solvent extracts.

b. Methanolic extract Aconitum heterophyllum Stem

TLC analysis of root extract in Chloroform and Methanol reports seven spots for various phytochemicals (Figure 1 (B)). The reported spots are separated with enough space and having various Rf values showing the presence of at least seven phytochemicals in Chloroform and Methanol solvent extracts.

c. Methanolic extract Aconitum heterophyllum Roots

TLC analysis of stem extract in Chloroform, Methanol and Ethyl acetate reports seven spots for various phytochemicals (Figure 1 (C)). The reported spots are separated with enough space and having various Rf values showing the presence of at least seven phytochemicals in Chloroform, Methanol and Ethyl acetate solvent extracts.

TLC profile of different extracts of Aconitum heterophyllum

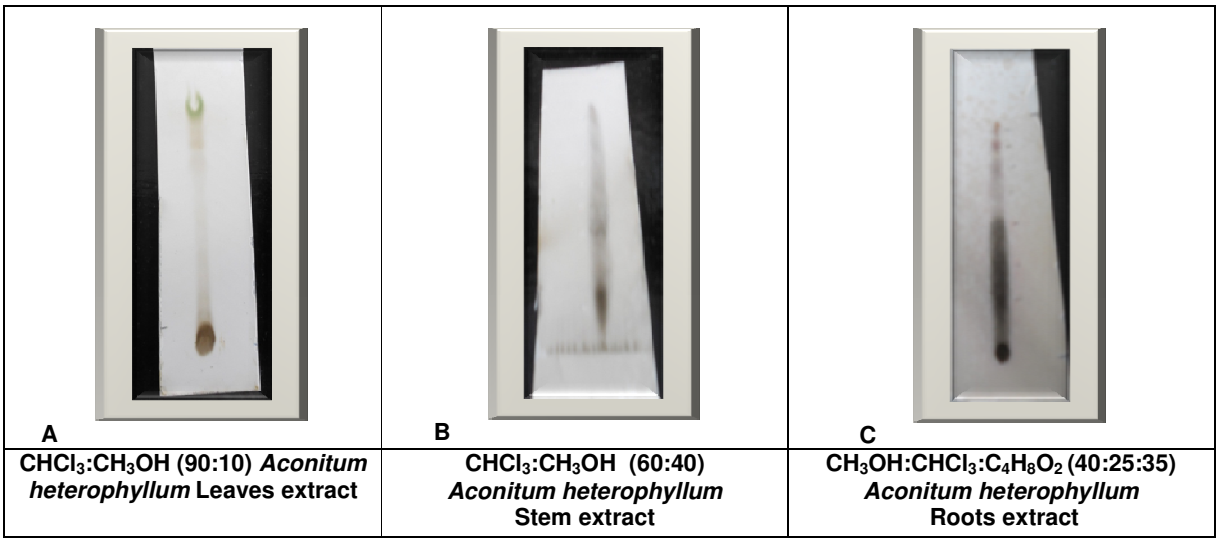

Figure1

TLC analysis shows the presence of different kinds of phytochemicals & Retention factor (Rfvalue) inleaves, roots, and stem extract.

DISCUSSION

When a new drug is to be discovered, qualitative phytochemical analysis is a very important step as it gives information about the presence of any particular primary or secondary metabolite in the extracts of the plant which is having a clinical significance. In any case, if any significant bioactive natural product is present, it is necessary to separate that compound from the mixture of compounds by using suitable chromatographic technique. The different phytochemical tests performed on the extracts of *Aconitum hetrophyllum*

leaves show the presence of alkaloids, carbohydrates, protein, amino acid, saponin, phenolic compounds, tannins, cardiac glycosides, flavonoids, Methanolic extract *Aconitum heterophyllum* Leaves. The different phytochemical tests performed on the extracts of *Aconitum hetrophyllum* roots show the presence of alkaloids, carbohydrates, protein, amino acid, saponin, phenolic compounds, tannins, steroids, cardiac glycosides, quinones, flavonoids, terpenoids. Methanolic extract and Ethyl acetate extracts reports the presence of alkaloids, steroids, cardiac glycosides, saponin, phenolic compound, carbohydrates, proteins, amino acid. Methanolic extract reports the presence of alkaloids, steroids, cardiac glycoside, saponin, Phenolic compound, carbohydrates, proteins, amino acid and in case of petroleum ether extracts alkaloids, steroids show their presence. The findings are also in line of previousfindings and reported literatures[13.] The different phytochemical tests performed on the extracts of *Aconitum hetrophyllum* stem showed the presence of alkaloids, carbohydrates, protein, amino acid, saponin, phenolic compounds, tannins, steroids,cardiac Glycosides, quinones, Flavonoids, terpenoids. Methanolic extract *Aconitum heterophyllum* stem. TLC profiling of all three extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals give different Rf values in different solvent system. This variation in Rf values of the phytochemicals provide a very important clue in understanding of their polarity and also help in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the Rf values of compounds in different solvent system. Different Rf values of the compound also reflect an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts[14].

CONCULUSION

The results obtained in the present investigation indicated *Aconitum heterophyllum* as a rich source of secondary metabolites. These findings suggested that *A. hetrophyllum* could be a potential source of natural antioxidant having great importance as therapeutic agent and preventing oxidative stress related degenerative diseases.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Deepak Kumar, H.O.D, Department of Pharmachemistry in addition to Management of Dolphin (P.G.) Institute of Biomedical and Natural Sciences, Dehradun, Uttarakhand, India for providing necessary research facilities. The Uttarakhand state biotechnology department (USBD) is thanked for financial assistance.

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