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Phytochemical studies on *Achyranthes aspera*

Abhaykumar Kamble

Nutrition and Medicinal Plants Lab, Department of Biochemistry, Gulbarga University, Gulbarga, Karnataka. India

E-mail address: abhay.gene@gmail.com

ABSTRACT

Phytochemical analysis was carried out on the plant *Achyranthes aspera* which revealed the presence of medicinally important bioactive compounds. The presence of phytochemical compounds in the plant *Achyranthes aspera* was evaluated in root using different solvents such as Petroleum ether, Chloroform, Methanol In the present study, the preliminary phytochemical screening of leaf and flower extracts of *Achyranthes aspera* showed the presence of phytochemicals such as alkaloids, carbohydrates, flavonoids, proteins, aminoacids, tannins, phenols, steroids, glycosides and saponins.

***Keywords*:** *Achyranthes aspera*, phytochemical, solvents

1. INTRODUCTION

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites and they are naturally synthesized in all parts of the plant body, bark, leaves, stem, root, flower, fruits, seeds etc. (ie) any part of the plant body contain active components (Criagg and David, 2001; Chhabra et al., 1984). The medicinal value of a plant lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants include alkaloids, tannins, carbohydrates, terpenoids, steroids and flavanoids (Edeoga, 2005). Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new sources (Geidam.,

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2007). Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Tiwari, 2011). This therefore underscores the need to try as much solvents as possible in screening plant parts for phytochemicals.

Plants are a tremendous source for the discovery of new products of medicinal value, which now-a-days are considered less risky than those of microbial and animal origin (Shanks and Morgan, 1999). The beneficial medicinal effects of plant material typically result from the endogenous combination of their secondary products. The progress of the genetic engineering domain along with medicinal plants has ascribed however to the increased yield in pharmaceutically important secondary metabolites (Briskin, 2000).

Achyranthes aspera is one of the plants used for medicinal purposes. It belongs to family Amaranthaceae. It is an erect, annual herb, distributed in hilly districts of India (T. Vetrichelvan et al, 2002). The plant is used in indigenous system of medicine as emenagogue, antiarthritic, antifertility, laxative, ecbolic, abentifacient, anti-helminthic, aphrodisiac, antiviral, anti-plasmodic, antihypertensive, anti-coagulant, diuretic and anti-tumor (Ratra et al, 1970). It is also useful to treat cough, renal dropsy, fistula, scrofula, skin rash, nasal, infection, chronic malaria, impotence, fever, asthma, piles and snake bites.

2. MATERIALS AND METHODS

Collection of plant material

Whole plants of *Achyranthes aspera* were collected from Gulbarga University Campus, Gulbarga. After collection the plant specimens were brought to the Department of Botany, Gulbarga University which was then identified as *Achyranthes aspera*.

Methods of extraction

Hot extraction method using soxhlet apparatus was used for successive solvent extraction of the harvested plant materials. The whole plants collected were shade dried to complete dryness and then the material was ground to fine powder in a mixer-grinder. The powder was weighed and 2 packets of approximately 35g of plant material was extracted successively using solvents ranging from polar to non-polar i.e. petroleum ether (40-60 ºC), chloroform (60-70 ºC), and methanol (80 ºC) in a soxhlet. After complete extraction, the contents of each extraction were concentrated by distillation. The concentrated extracts were evaporated to dryness and weighed.

Petroleum ether yields a greenish brown gummy extract, chloroform yields a dark green powder extract, where as methanol yields light greenish soft powder extract.

Phytochemical studies

The extracts obtained after successive solvent extraction were qualitatively tested for the presence of various phytochemicals. The preliminary phytochemical screening was carried out as described by Harborne (1991) and Kokate (1995).

Preparation of reagents for phytochemical test.

- **Molisch's reagent:** 10g of alpha-naphthol dissolved in 100ml of 95% ethyl alcohol.

- **Anthrone reagent:** 0.2g of anthrone dissolved in 100 ml of conc. H_2SO_4 and shaken well (the solution to be freshly prepared)
- **Biuret reagent**: 1.5g of $CuSO_4$, 6g of sodium potassium tartrate was dissolved in 500 ml of water, 300 ml of freshly prepared NaOH solution was added to this solution made up to volume of 1 liter.
- **Ninhydrin reagent:** 0.1% Ninhydrin dissolved in 100 ml of acetone.
- **Dragendroff's reagent:** 50 mg of tartaric acid was dissolved in 200 ml of distilled water, 4.5g of basic bismuth nitrate was added and solution was shaken for 2 hours. 100 ml of 40% potassium iodide was then added and solution was shaken vigorously and allowed to stand for 24 hours and filtered.
- **Mayer's reagent:** 1.3g of mercuric chloride and 5g of potassium iodide were dissolved separately in 60 ml and 10 ml of distilled water respectively. Both were mixed vigorously and diluted up to 100 of distilled water.
- **Phenolphthalein reagent:** 1g of phenolphthalein dissolved 100 ml of distilled water.
- **Lead Acetate (10%):** 10g of lead acetate dissolved in 200 ml of distilled water.
- **5% Sodium Nitrate solution:** 5g of $NaNO_3$ was dissolved in 100 ml of distilled water.
- **Ferric Chloride solution 5%:** 5 mg of Ferric chloride was dissolved in 100ml of distilled water.
- **0.1N Alcoholic KOH:** 5.6g of Potassium hydroxide dissolved in 100 ml of ethyl alcohol.
- **Gelatin containing 10% NaCl:** 1g of gelatin dissolved in 100 ml of 10% NaCl.
- **Hydrochloric acid:** 50 ml of concentrated HCl dissolved in 50 ml of distilled water.
- **Aqueous NaOH 10%:** 10g of sodium hydroxide dissolved in 100 ml of distilled water.
- **Copper Sulphate solution:** 24.9g of copper sulphate dissolved in 100 ml of distilled water.
- **Iodine in Potassium Iodide:** Dissolve 20g of potassium iodide and 12.7g of iodine in 30 ml of distilled water and diluted to one liter.
- **Conc. H_2SO_4:** specific gravity 1.84

Phytochemical screening

1. **Test for Alkaloids** (Evans 1997):
   Alkaloids are basic nitrogenous plant products that are most optically active and possess nitrogen. They have heterocyclic structural units with pronounced physiological action.

i. **Mayer's Test**: 1ml extract + 4-5 ml of dilute HCl shake well and add Mayer's Reagent, formation of white or pale yellow precipitate indicates the presence of Alkaloids.

ii. **Dragendroff's Test**: 1ml extract + 4-5 ml of dil HCl shake well and add Dragendroff's Reagent, formation of orange precipitate indicates the presence of Alkaloids.

iii. **Wagner's Test**: 1ml extract + 4-5 ml of dil HCl shake well and add Wagner's Reagent, formation of brown precipitation indicates the presence of Alkaloids.

2. **Test for Phenolics** (Gibbs, 1974):

Phenols are aromatic compounds with hydroxyl groups that are widely spread in plant kingdom. They occur in all parts of the plant. These offers resistance to diseases in plants .Grains contain high amount of polyphenols which are resistance to bird attack.

i. **Phenol Test**: 1 ml extract+ Ferric chloride solution, formation of yellow precipitate indicates the presence of phenols.

ii. **Ellagic acid Test**: 1 ml extract + few drop of 5% (v/v) Glacial acetic acid + 5% (w/v) sodium carbonate solution. The solution turns to muddy yellow, olive brown, niger brown and chocolate colour indicates the presence of phenols.

iii. **Hot water test:** Dip a leaf mixture in the test tube containing hot water, warm it for few minutes. The development of black or brown colour ring at the junction of dipping indicates the presence of phenols.

3. **Test for Tannins** (Trease and Evans, 1989)

i. **Ferric Chloride Test:** 1 ml extract + 1% Ferric chloride solution. Formation of blue green or brownish green colour indicates the presence of Tannins.

ii. **Gelatin test:** Extract + 3 drops 1% solution of gelatin containing 10% NaCl. Foramtion of white precipitation indicates the presence of Tannins.

4. **Test for Saponins** (Kokate, 1999):

These are plant steroid compounds or triterpeniods which are identified by their bitter taste ability. They form foam in aqueous solution and lyse erythrocytes.

i. **Foam Test**: 1 ml extract + Shaken well with water. Formation of honey comb like foam indicates the presence of Saponnins.

ii. **Blood Test**: 1 ml extract + few drops of blood and observe under microscope. The lyse of blood cells was observed under microscope indicates the presence of Saponins.

5. **Test for Flavoniods** (Iyenger 1995)

These are also known as Anthoxanthins that are yellow pigments which occur in plant kingdom.

i. **Flavoniodes Test**: 1 ml extract + few magnesium turnings + conc. H_2SO_4 dropped through the sides of tube. Formation of magneta colour, scarlet colour, deep cherry colour indicates the presence of Flavonols, Flavones and Flavoniods.

ii. **Ferric chloride Test**: 1 ml extract + Neutral Ferric chloride solution. Formation of blackish vgreen colour indicates the presence of Flavoniods.

iii. **Lead Acetate Test**: 1 ml extract + Lead acetate solution. Formation of yellow precipitate indicates the presence of Flavoniods.

iv. **Shinoda Test:** 1 ml extract + Conc. HCl + Few magnesium turnings. Formation magenta Colour indicates the presence of Flavonone/Flavone.

v. **Zinc-Hcl Reduction Test/ Pew's Test:** 1ml extract + pinch of zinc powder + few drops of 5N HCl. Formation of purple, cherry red and pink or brownish colour indicates the presence of Flavonoids.

6. **Test for Sterols** (Gibbs 1974)

These are of large class of organic compounds occurring widely in plants and animals and are characterized by the presence of 1,2-cyclopentanophenenthrane ring system which may be partially deduced or other wise modified. Examples: Steroids, Bile salts, Adenocarticoids etc.

i. **Salwoski Test**: 1 ml extract + Conc. H_2SO_4. Formation of wine red colour indicates the presence of Sterols.

ii. **Libermanm- Buchard's Test**: 1 ml extract + acetic Anhydride + Conc. H_2SO_4 along the sides of tube. Formation of red ring at the junction of two layers indicates the presence of Sterols.

7. **Glycosides** (Ramakrishnan et al., 1994)

Heamacetyl form of a sugar reacts with a molecule of an alcohol to form the acetyl derivatives which are generally known as glycosides. Those of sugars known as glucosides or fructosides.

i. **Keller- kilani Test**: 1 ml extract + mixed with few drops of glacial acetic acid and boiled for a min and cooled. To this solution add 2 drops of ferric chloride solution. The contents were transferred to another tube containing Conc. Sulphuric acid. Formation of reddish brown ring at the junction of 2 layers indicates presence of Glycosides.

ii. **Molisch's Test**: 1 ml extract + Molisch reagent + 1 ml Conc. sulphuric acid along the sides of the tube. Foramtion of Reddish violet ring at the junction of 2 layers indicates the presence of Glycosides.

8. **Carbohydrate Test** (Ramakrishnan et al., 1994)

These are the substances with general formula of C_x (H_2O) are called as carbohydrates which contains hydrogen and oxygen in the same proportion as in water.

i. **Molisch's Test**: 1ml Extract + Molisch Reagent. Add 2 ml of conc. H_2SO_4 along the sides of the test tube of the walls and allow it stand for 2 mins. Formation of reddish violet colour at the junction of two layers, indicates the presence of carbohydrates.

9. **Amino acid and Protein Test** (Fischer, 1968 and Ruthmann, 1970)

Proteins are complex nitrogenous compounds which occur in plant and animal cells. Proteins on hydrolysis with strong inorganic acids or by enzymes yield a mixture of amino acids.

i. **Ninhydrin Test**: 1 ml extract + Ninhydrin reagent heat for 2-3 mins, formation of purple colour indicated the presenec of Amino acids.

Thin Layer Chromatography (TLC) Separation

For the qualitative detection of alkaloids in the extracts, the extracts were run on TLC glass slides made up of silica gel G slurry. Glass slides of size 6×2.5 cm were thoroughly cleaned with tap water and wiped with acetone in the end and allowed to dry Silica gel G slurry was prepared in distilled water and then poured over the slides to form TLC plates of uniform thickness.

The freshly prepared plates were then allowed to dry completely and heated to activate in oven at about 80 °C for ½ hour. The plant extracts were then loaded on the TLC plates with the help of capillary tubes and air dried.

The TLC plates were run in solvent systems for separation of extracts on the plate. Separation occurs based on partition principle between mobile phase and stationary phase. The mobile phase selected was methanol: chloroform (3:7).

It was poured in a glass developing chamber and kept covered with lid for half an hour for saturation of chamber. Then the TLC plates loaded with extract were placed vertically in the chamber with solvent. Once the solvent front moves upto ¾th or edge of other end, the plates were taken out and air dried. Both the ends of the solvent run was marked with a glass marker and then the plates were sprayed with Dragendroff Reagent and again air dried.

After drying, the development of brick red colour on the plate indicates presence of alkaloids.

Relative front or R_f value of the separated components can be calculated as follows:

$$R_f = \frac{Dis\,tan\,ce\ traveles\ by\ the\ solute}{Dis\,tan\,ce\ traveles\ by\ the\ solvent} \times 100$$

HPTLC Separation

An attempt was made to know about the nature of the constituents and their proportion present in the extracts derived by successive hot extraction. For this, dried samples of different extracts (Petroleum ether, Chloroform and Methanol) were sent for High Performance Thin Layer Chromatography analysis at Anchrom Enterprises Pvt. Ltd., Mumbai for detection of saponins and Alkaloids.

3. RESULTS

Phytochemical Screening of *Achyranthes aspera*

The extracts obtained from successive hot extraction of plant parts were subjected to different phytochemical tests to reveal the presence of different phytochemicals more importantly secondary metabolites present in the extracts.

1. **Carbohydrates:** The leaf extracts of petroleum ether, chloroform and methanol responds positively to Molish and Anthrone test where as the other flower extracts showed negative results
2. **Protein and Amino acids:** All the extracts responded negatively to Ninhydrin acid indicating absence of protein in the extracts
3. **Steroids:** only leaf and flower extracts of petroleum ether and leaf extract of methanol displayed positive response to all the other extracts showed negative result
4. **Phenolic compound:** Phenolic compound was detected by Ellagic acid test and phenol test to be present in only the leaf extract of methanol and absent in the rest.
5. **Glycosides:** Tests for glygosides by Kellar Kiliani and Sulphuric acid test revealed presence in leaf and flower extracts of petroleum ether, leaf extracts of chloroform and methanol and absence in other extracts
6. **Saponin:** foam test for detection of saponin revealed presence of saponins in flower extracts of petroleum ether and chloroform and leaf extract of methanol only the other extracts responded negatively to the foam test
7. **Tannins:** leaf extracts of all the three solvents and flower extract of methanol responded positively to $FeCl_3$ test
8. **Alkaloids:** Drangendroff test and Mayer's test revealed the presence of alkaloids in only the leaf and flower extracts of methanol where as absence in petroleum ether and chloroform extracts.
9. **Flavonoids:** lead acetate test revealed absence of flavonoids in all the extracts

Table 1. Phytochemical screening of *Achyranthes aspera*

SL.NO	TEST	PETROLEUM ETHER		CHLOROFORM		METHANOL	
		Leaf	Flower	Leaf	Flower	Leaf	Flower
1	Carbohydrate: Molisch Test Anthrone Test	 + +	 – –	 + +	 – –	 + +	 + +
2	Protein and Amino acids: Ninhydrin Test	 –	 –	 –	 –	 –	 –

3	Steroids: Salkowski Test Leibermann Burchard's reaction	 + +	 + +	 – –	 – –	 + +	 – –
4	Phenolic compound: Ellagic acid test Phenol Test	 – –	 – –	 – –	 – –	 + +	 – –
5	Glycosides: Kellar Kiliani Test Sulphuric acid Test	 + +	 + +	 + +	 – –	 + +	 – –
6	Saponin: Foam Test	 –	 –	 –	 +	 –	 –
7	Tannins: $FeCl_3$ Test	 +	 –	 +	 –	 +	 +
8	Alkaloid: Dragendroff Test Mayer's Test	 – –	 – –	 – –	 – –	 + +	 + +
9	Flavonoids: Lead acetate Test	 –	 –	 –	 –	 –	 –

TLC separation of plant extracts

The plant extracts was loaded in minor quantities on the TLC glass slides (6×2.5 cm) and partitioned using the mobile phase methanol: chloroform (30:70). Then they were sprayed with Drangendroff reagent and air dried. Formation of a red spot reveals presence of alkaloid which was seen only in the methanolic extract, thus confirming that alkaloids were present only in methanolic extract.

HPTLC Analysis of plant extracts

HPTLC analysis of the plant extracts was done for alkaloid and saponins to qualitatively detect their presence and estimate the proportion of their presence in the extracts.

- Analysis reveals the presence of alkaloids in the methanolic extract of *Achyranthes aspera*. By comparing with the peaks of alkaloid standard Achyranthine (R_f 0.65) and Betaine (R_f 0.88), we came to know that both achyranthine and Betaine were present in leaf extract and only Achyranthine was present in the flower extract of methanol.
- Also the HPTLC analysis for saponins was done using a standard 20-hydroxyecdysone. By comparing that this compound is majority present in flower extracts of petroleum ether, chloroform and methanolic extracts some minor saponins may also be present in these extracts other than the standard used.

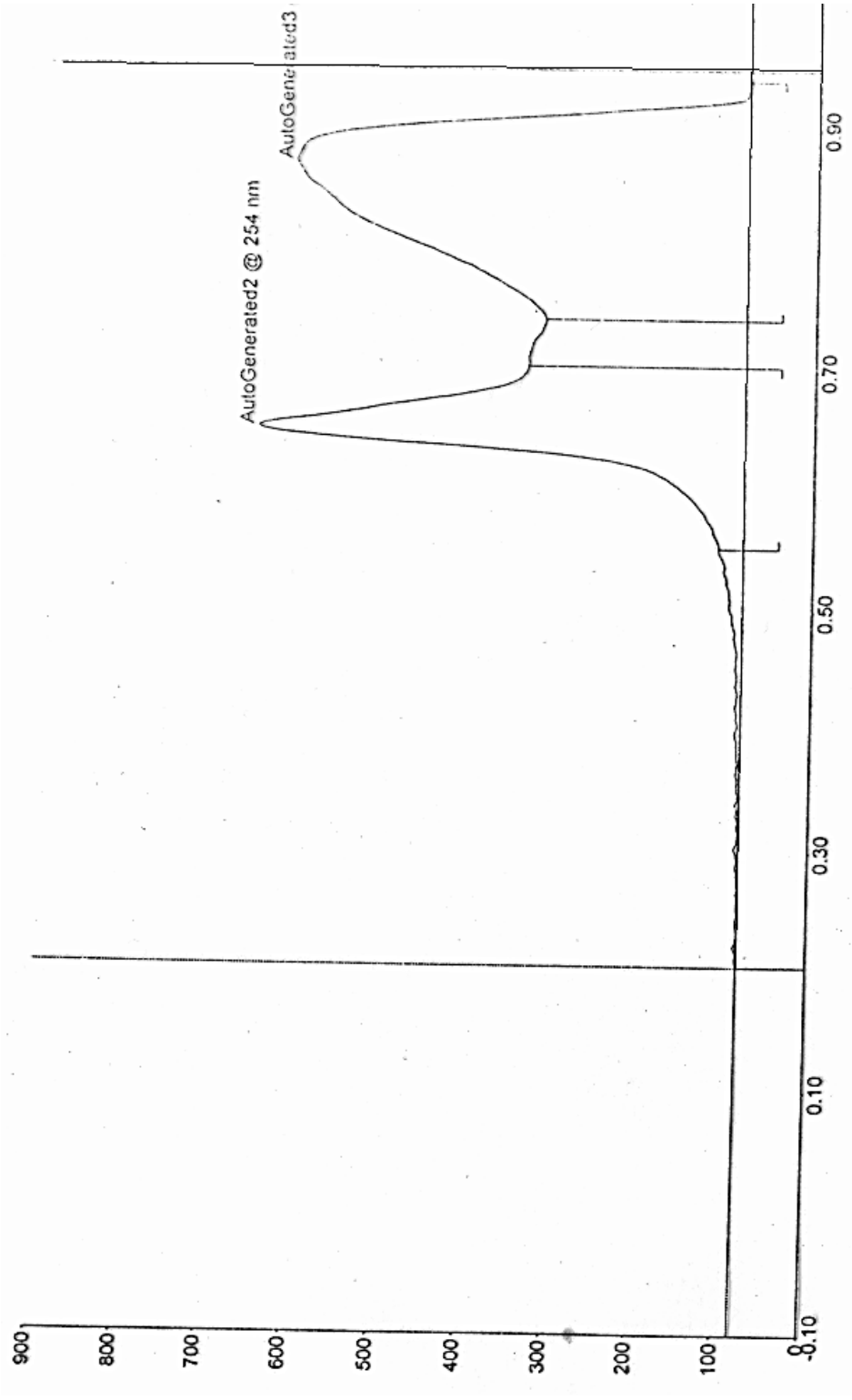


Fig. 1. HPTLC graph showing standard Alkaloid (Achyranthein, Betaine)

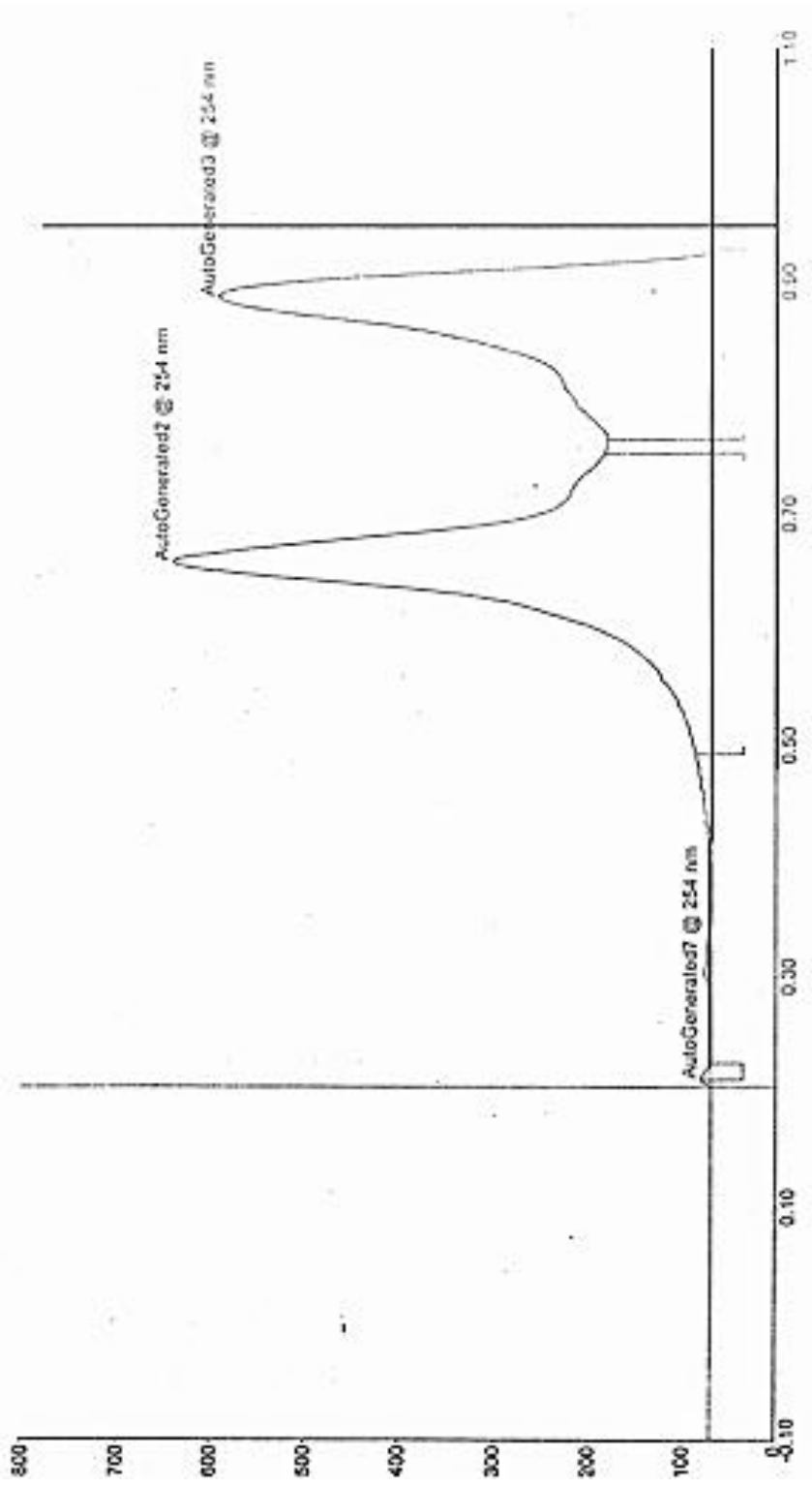


Fig. 2. HPTLC Graph showing presence of Alkaloid (Achyranthein, Betaine) in Methanolic Extract of *Achyranthes aspera* (Leaf)

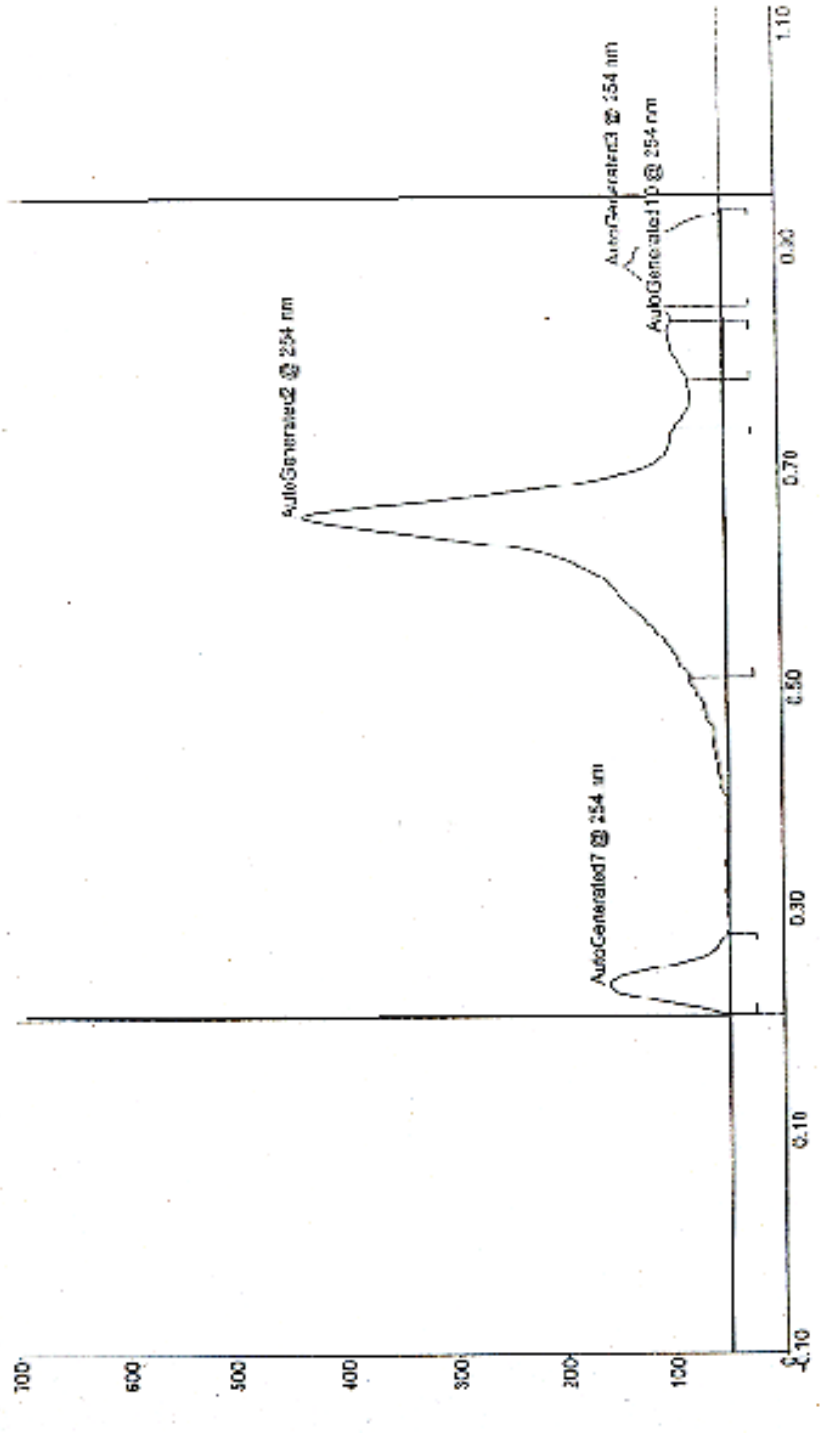


Fig. 3. HPTLC Graph showing presence of Alkaloid (Achyranthein) in Methanolic Extract of *Achyranthes aspera* (Flower)

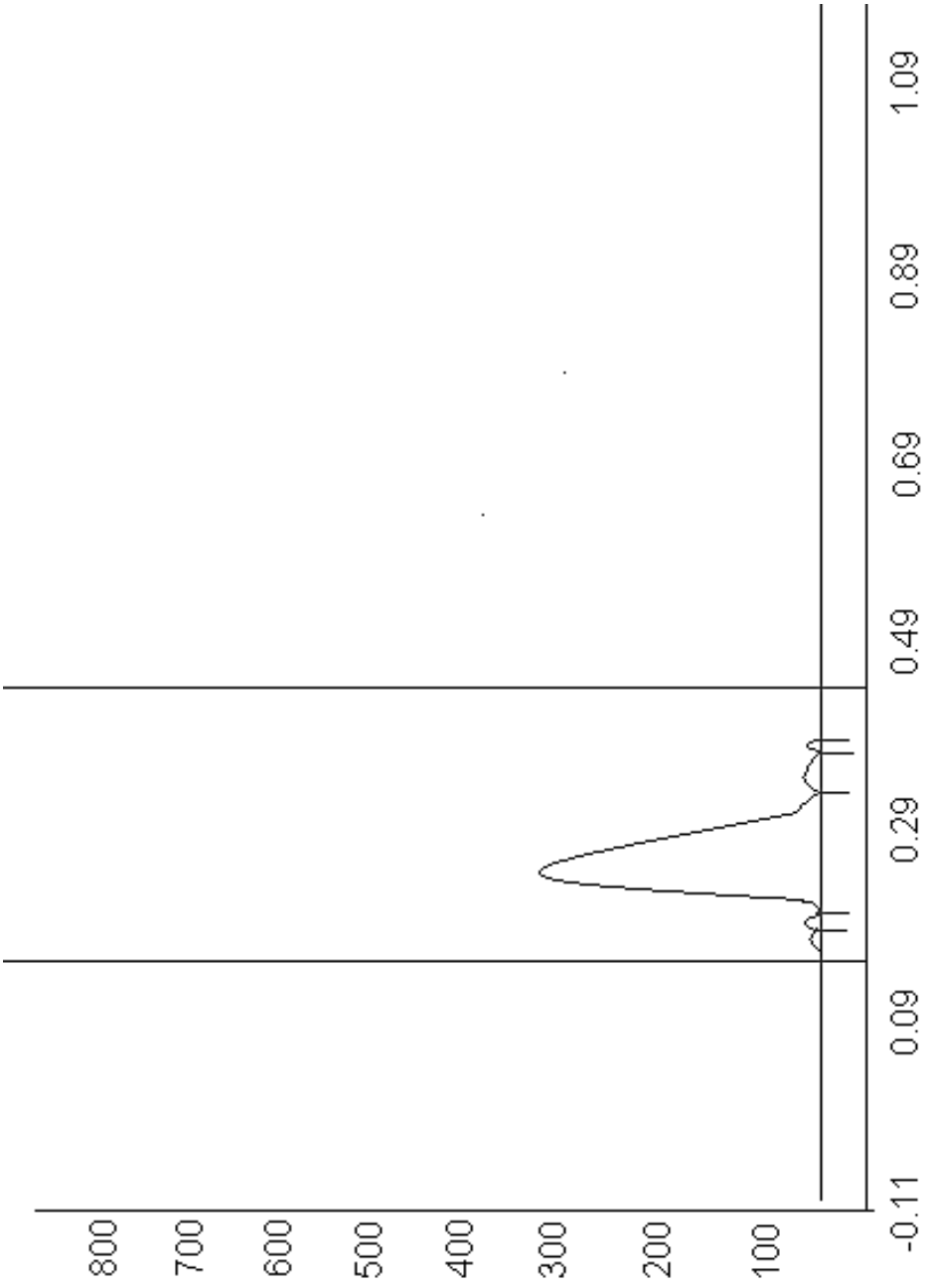


Fig. 4. HPTLC Results showing Standard Saponin (20-Hydroxyecdysone)

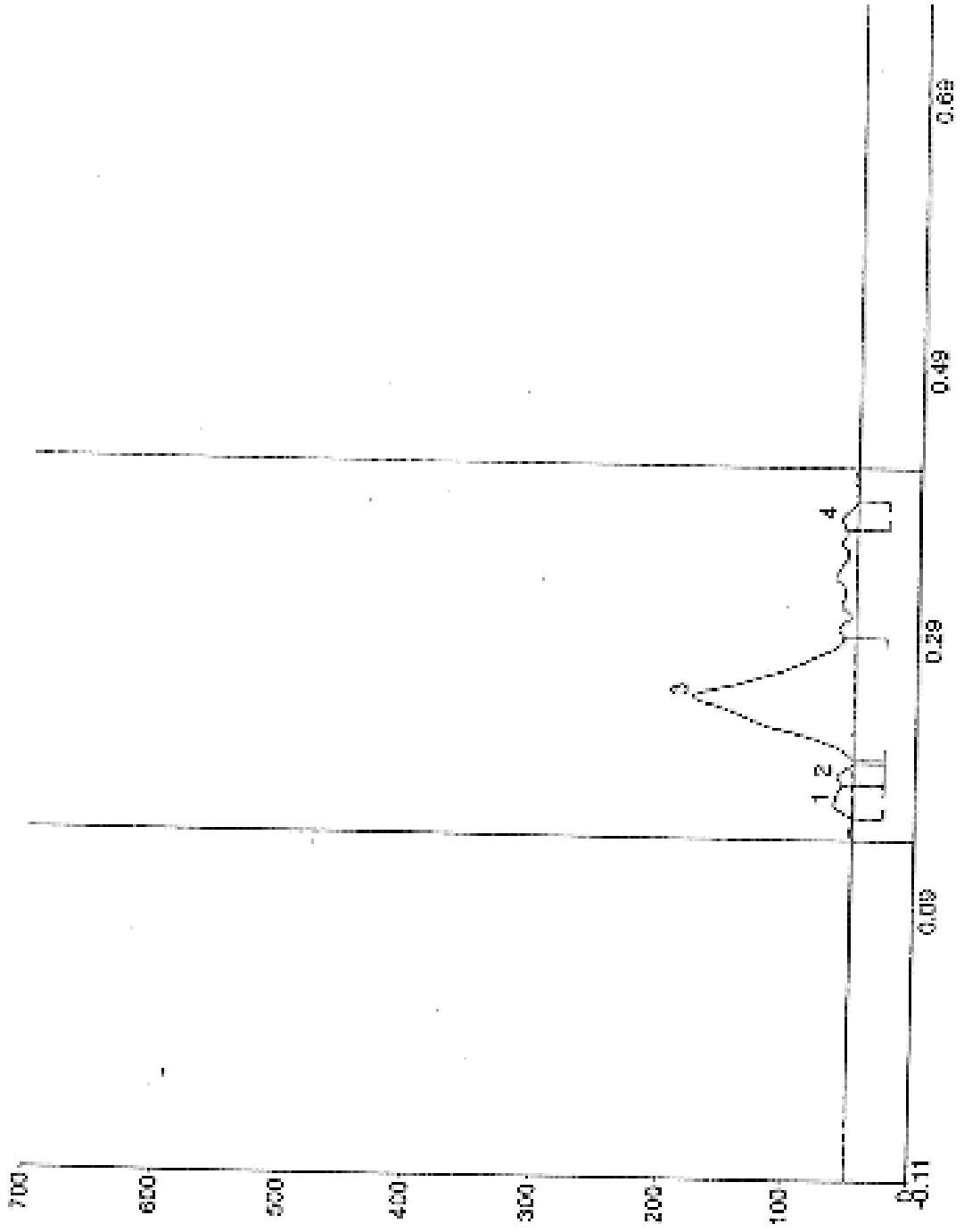


Fig. 5. HPTLC Result showing presence of Saponin (20-Hydroxyecdysone) Petroleum ether extract of *Achyranthes aspera* (Flower)

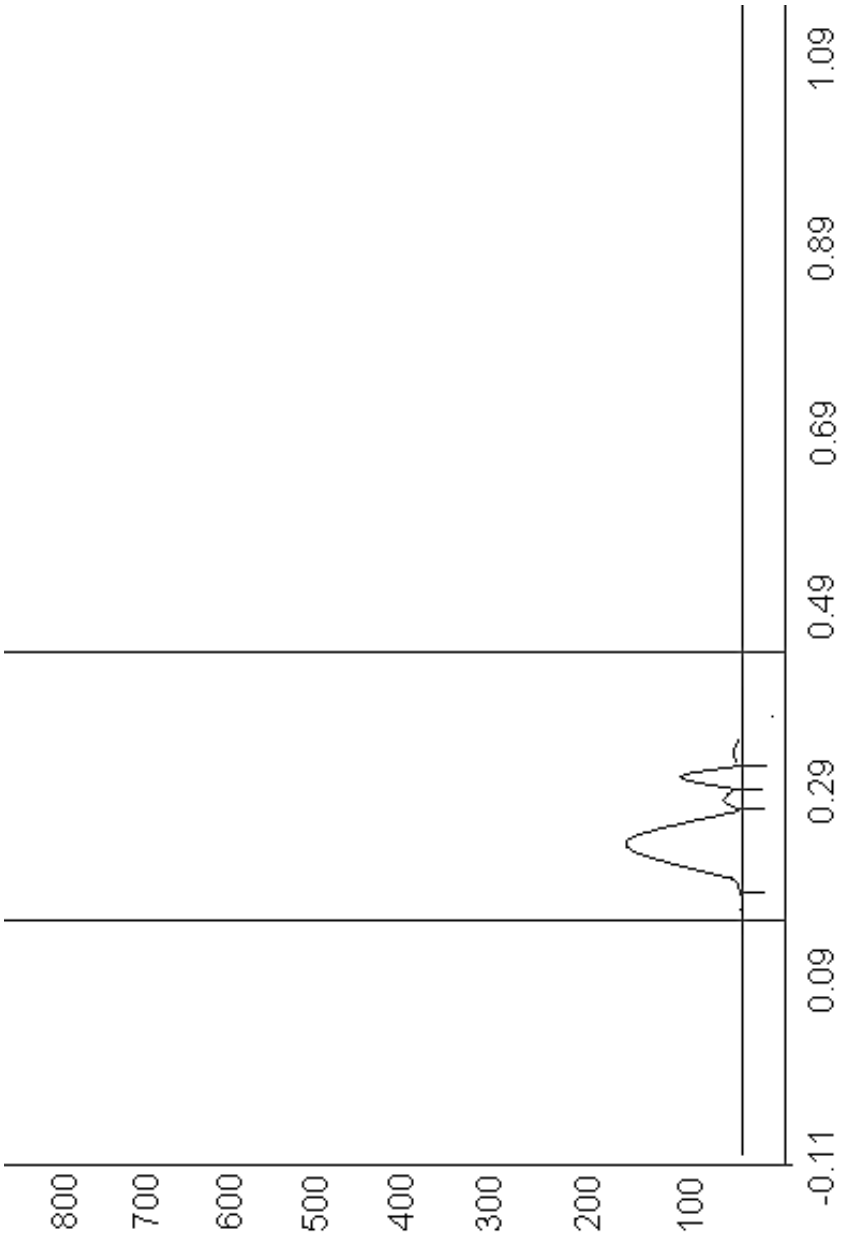
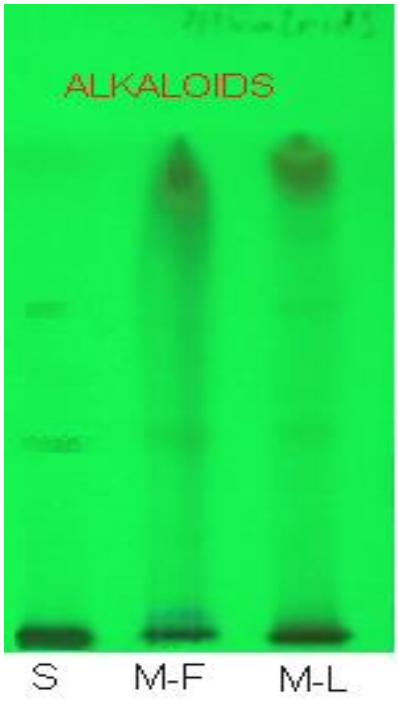
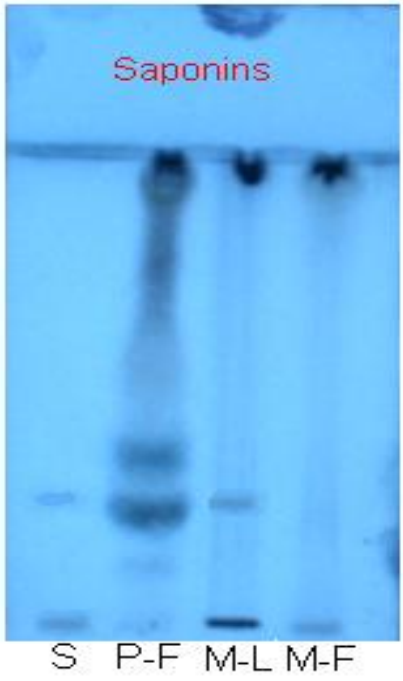


Fig. 6. HPTLC Result showing presence of Saponin (20-Hydroxyecdysone) in Methanolic extract of *Achyranthes aspera* (Leaf)



HPTLC images showing presence of Alkaloids



HPTLC images showing presence of Saponin

PHOTOGRAPH

4. DISCUSSION

Phytochemical screening presence of alkaloid was detected in methanolic extract of the plant. Along with this presence of glycosides and tannins were seen in petroleum extract, chloroform and methanolic extract. Saponins and steroids were present in petroleum ether and methanolic extract but flavanoids were absent in all extracts. Also phenolic compound was present in methanolic extract. All these, compounds may confirm the clinical affect associated with *Achyranthes aspera*.

The study reveals that wide numbers of phytochemical constituents have been isolated from the plant which possesses activities like antiperiodic, diuretic, purgative, laxative, antiasthmatic, hepatoprotective, anti-allergic and various other important medicinal properties. The plant is used in indigenous system of medicine as emenagogue, antiarthritic, antifertility, laxative, ecbolic, abentifacient, and antihelminthic, aphrodisiac, antiviral, anti-plasmodic, and antihypertensive, anticoagulant, diuretic and anti-tumor. It is also useful to treat cough, renal dropsy, fistula, scrofula, skin rash, nasal, infection, chronic malaria, impotence, fever, asthma, piles and etc.

T.N. Misra et al. (1996) isolated various compounds like tetracontanol-2 ($C_{40}H_{82}O$, melting point 76-77 °C), 4-methoxyheptatriacont-1-en-10-ol ($C_{38}H_{76}O$) and β-sitosterol. A. Banerji et al. (1971) isolated ecdysterone from the whole plant.

K.S. Laddha (2005) et al. reported extraction, isolation and purification of 20-hydroxyecdysone from Achyranthes aspera and its characterization by DSC, UV, IR, CD, 1H and ^{13}C NMR, MS and quantification by HPLC.

V. K. Kapoor & H. Singh (1966) reported betaine ($C_5H_{11}NO_2$) (m.p. 292 °C) from the whole plant which is also a water soluble alkaloid. The identity of betaine was confirmed by mixed m.p. detection of the HCl-salt, oxalate and picrate derivatives and compared with those of an authentic sample.

V. Seshadri et al. (1981) isolated two constituents from the fruits and were identified as Saponins C and D.

M. Ali (1993) isolated various compounds from the stem, Pentatriaontane, 6-pentatriacontanone, Hexatriacontane and Tritriacontane .

O. Kunert. et al. (2000) reported three bisdesmosidic saponins (I-III), 20-hydroxyecdysone, and quercetin-3-O-β-D-galactoside, were isolated from the methanol extract of the aerial parts of Achyranthes aspera. Their structures were established on the basis of NMR spectroscopic analysis; the complete ^{1}H and ^{13}C assignments of the compounds were achieved by means of ^{2}D NMR studies.

5. CONCLUSION

The present study suggests that the extracted phytochemicals are very valuable. Furthermore, isolation, purification and characterization of the phytochemicals will make interesting studies. Further investigations are planned to conduct the pharmacological studies to know the potency of these extracts. The parts of achranthus aspera are used in traditional systems of medicines, seeds, roots and shoots are the most important parts which are used medicinally. The major chemical constituents are carbohydrates, protein, glycosides, alkaloids, tannins, saponins, flavoides, lignin etc.

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