Phytochemical screening and Thin layer chromatographic identification of Terpenoids from the root extract of Achyranthes aspera l.- An Indian Ethanomedicine

Asmita Singh[*1], .Manisha Mathur[2], Ajay[2] kumar,.Gajendra Pal Singh
Department of Botany, University of Rajasthan, Jaipur, Rajasthan
Indian Veterinary Research Institute, Bareilly, U.P
Email:asmitaivri@gmail.com

Abstract: Medicinal plants are big source of information for wide variety of chemical constituents which could be developed as drug with precise selectivity. *Achyranthes aspera* Linn is one of the important medicinal plants having many therapeutic uses. The present study deals with preliminary phytochemical screening and TLC investigation of *Achyranthes aspera Linn.* In qualitative analysis, the phytochemical compounds such as steroids, triterpenoids, sugars, alkaloids, phenolic compounds, flavonoids were screened in root methanolic extract by using standard methods. Phytochemical screening of plant revealed the presence of alkaloids, flavonoids steroids reducing sugar, glycosides and Terpenoids. The present study revealed that *Achyranthes aspera* roots are important source of many therapeutically and pharmacologically active constituents.

Keywords: *Achyranthes aspera*, Terpenoids ,Phytochemical analysis, Flavonoids,Triterpenoids ,Therapeutically

I.INTRODUCTION

Ethanomedicinal study deal with the study of traditional medicine .since ancient times mankind has been using herbal plants, organic materials from the sea, rivers etc .for its betterment. Recently much attention has directed towards extracts and biologically active isolated from popular plant species .In the present era of drug development and discovery of newer drug molecules ,many plant products are evaluated on the basis of their bioactive constituents .The curative properties of medicinal plants are mainly due to presence of various complex chemical substances of different compositions which occur as secondary metabolites.(Karthikeyan et.al.,2009)The therapeutic properties of medicinal plants are mainly due to the existence of an assortment of complexchemical substances of diverse compositions which occur as secondary metabolites.The most significant of theses bioactive constituents are alkaloids ,glycosides ,flavanoids ,tannin are terpenoids.various parts of the plants like roots ,leaves ,bark ,exudates etc are used as per medicinal propertites(Perumalet.al.,2007).They are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design(Akinjogunia et.al,2010).*Achyranthes aspera Linn* belongs to the family Amaranthaceae.It is an annual stiff erect herb and found commonly as a weed throughout india and is one of the important medicinal plants having many therapeutic use as Odontalgic ,Rheumatism, Bronchitis ,skin disease and rabies(Girach et.al1992)·Leaf extract were reported to posses thyroids stimulatingandanti-peroxidativeproperties(Tahilianiet.al2000).The aqueous and methyl alcohal extracts of plant also decreased blood glucose levels in normal and alloxan diabetic rabbits(Akhtar et.al1991) .The main aim of the present investigation was to study the phytochemical constituents and identification of two pharmaceutically valuable terpenoids from methanolic extract of roots of *Achyranthes aspera.*

I. MATERIAL AND METHOD

1.Collection and identification of plant material

The specimen was collected from University Botanical Garden,Departmentof Botany, University of Rajasthan, Jaipur.The roots of the plant*(Achyranthes aspera)*were washed thoroughly 2-3 times with running tap water and then with sterile distilled water .air dried at room temperature .After complete drying roots were powdered well using a mixer. Powdered samples were extracted through soxhlet extraction with methanol. The crude extract were collected in amber coloured sample bottles and stored .All chemical and reagents used including the solvents were of analytical grade.

Phytochemical Analysis

Phytochemical analysis was carried out in the methenolic extract of the roots of Achyranthes aspera using standard procedures to identify constituents as described by Harbone(1984),Trease and Evans (1979) and Sofowara (1993).

Test for alkaloids

Methanolic extract was warmed with 2% H_2SO_4 for two minutes .it is filtered and few drops of reagents were added and indicated the presence of alkaloids .

a)Dragendroff's test

To 5 ml of extract few drops of Dragendroff's reagent was added for the formation of orange coloured precipitate. A orange precipitation indicates the positive.

b)Mayer's test

To 5 ml of extract few drops of Mayer's reagent was added for the formation of creamy-white coloured precipitate .This precipitation indicates positive .

c)Wagner's test

To 5 ml of extract few drops of Wagner's reagent was added for the formation of Reddish brown coloured precipitate .this reddish brown coloured precipitation indicate positive .

d)Hager's test

To 3 ml of extract few drops of Hager's reagent (Picric Acid (1%)- was added for the formation of prominent yellow colored precipitate .this yellow colored precipitation positive.

Test for Flavonoids

A small quantity of extracts is heated with 10ml of ethyl acetate in boiling water for 3 minutes .The mixture is filtered differently and the filtrates are used for the following test

a)Ammonium Test

The filterate was shaken with 1 ml of dilute ammonia solution (1%).The layers were allowed to separate .A yellow coloration was observed at ammonium layer. This Indicates the presence of flavonoids .

b)Aluminium Chloride Test

The filtrates were shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow color .it indicated the presence of Flavonoids and diluted NaOH and HCL was added .A yellow solution that turns colorless indicates positive.

Test for Terpenoids

a)Salkowski Test

The extract was mixed with 2ml of chloroform and concentrate H2SO4 (3ml) is carefully added to form a layer.A reddish brown coloration of the interface is formed to show positive result of presence of terpenoids .

Test for Tannins

A small quantity of extract is boiled with 5 ml of 45%solution ethanol for 5 minutes .each of the mixture is cooled and filtered.filtrates were used to the following test .

a)Lead Sub Acetate Test

1ml of the different filtrate was added with three drops of lead sub acetate solution. A cream gelatinous precipitation indicates positive test for Tannins.

b)Ferric Chloride Test

1ml each of filtrate is diluted with distilled water and added with two drops of ferric chloride. A transient greenish to black color indicates the presence of Tannins.

Test for sterols

a)Liebermann-Burchard test

To a small amount of the extract few drops of chloroform, acetic anhydride and H2SO4 was added along the sides of the test tube to observe the formation of dark red or pink colour.

Test for proteins

a)Ninhydrin test

To the test solution added 1 ml of 0.2 % ninhydrin solution, violet color indicate the presence of protein in sample

b)Biuret test

To 3 mL of the extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple color. On addition of alkali, it becomes dark violet.

c) Xanthoprotein test

To 3 mL of the extract few drops of HNO3 reagent was added for the formation of intensely yellow colour.

Test for carbohydrates

a)Molisch's test

To a small amount of the extract few drops of Molisch's reagent was added followed by the addition of conc. H2SO4 along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 mL of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates.

b)Fehling's test

The extract was treated with 5 ml of Fehling's solution (A and B) and kept in boiling water bath. The formation of yellow or red color precipitate indicates the presence of reducing sugar

Test for Glycosides

5ml of diluted sulphuric acid was added in extracts in a test tube and boiled for fifteen minutes in a water bath. It was then cooled and neutralized with 20% potassium hydroxides solution. A mixture of 10ml of equal parts of Fehling's solution A and B were added and boiled for five minutes. A more dense red precipitate indicates the presence of glycosides

a)Baljet's Test

To 5 mL of the extract few drops of sodium picrate was added to observe yellow to orange colour.

b)Keller-Killiani test

To 5 mL of the extract few drops of ferric chloride solution was added and mixed, then sulphuric acid containing ferric chloride solution was added, it forms two layer showed reddish brown while upper layer turns bluish green indicates the presence of glycosides

Test for saponins

a)Foam test

To a small amount of the extract few drops of distilled water was added and shaken vigorously until persistent foam was observed.

TLC identification of two terpenoids from methanolic root extract:

Phytochemical identification of terpenoids from extract by thin layer chromatography was performed as per the method .Briefly ,the extract were drawn into capillary tubes and applied as spots on a stationary phase (silica gel coated plate)about 1 cm from the base. The plates was then dipped into a suitable solvent system (mobile phase) and placed in a well covered tank.

Chromatographic tank was saturated with mobile phase at room temperature for 5 min prior to development. After that the plates were removed dried and processed for the identification of separated compounds(as colored spots) and the Rf values were calculated using the formula .

Rf value =(Distance moved by the compound)/Distance moved by the solvent front)

III.RESULT AND DISCUSSION

Extraction of Plant Material

The roots of the plant*(Achyranthes aspera)*were washed thoroughly 2-3 times with running tap water and then with sterile distilled water .air dried at room temperature .After complete drying roots were powdered well using a mixer. Powdered samples were extracted through soxhlet extraction with methanol. The crude extract were collected in amber coloured sample bottles and stored .All chemical and reagents used including the solvents were of analytical grade.

Figure 1: Achyranthes aspera L. Roots

Phytochemical Analysis

Powdered roots of *Achyranthes aspera* were subjected to various qualitative tests for the identification of phytochemical constituents includes tests for alkaloids (Dragendroff's test, Mayer's test, Hager's test, Wagner's test), saponins,glycosides(Baljet's test, Kellar-Killianitest), carbohydrates (Molisch's test, Fehling's test), proteins (Biuret test, Xanthoprotein test), tests for tannins, flavonoids, steroids (Liebermann-burchard test), phenols, terpenoids were performed using specific reagents and results are tabulated in Table 1.

Table no -1 preliminary phytochemical screening of methanolic extract of roots of achyranthes aspera linn.

Sl. No	Test for	Reagents	Reaction	Inference
1	Alkaloids:	Dragendroff's Reagent Mayer's reagent Wagner's reagent Hager's reagent (Picric Acid (1%)-	Orange precipitation Creamy-white precipitation Reddish brown precipitation yellow coloured precipitate	+++
2	Flavanoids	Dilute ammonia solution (1%).	Yellow coloration was observed at ammonium layer	++
3	Carbohydrates:	Molisch Reagent Copper sulphate+ potassium sodium tartarate +NaOH	Formation of red or dull violet colour at the inter phase Formation of yellow or red color precipitate	+
4	Terpenoids	2ml of chloroform and concentrate H2SO4 (3ml) (Lieberman Test)	Reddish brown coloration of the interface	+++
5	Steroids	Few drops of chloroform, acetic anhydride and H2SO4(salkowski Test)	Dark red or pink colour	+
6	Glycosides	Few drops of sodium picrate Few drops of ferric chloride solution	Observe yellow to orange colour Reddish brown while upper layer turns bluish green	+
7	Proteins and amino acids	0.2 % ninhydrin solution Few drops of HNO3 reagent	violet color Formation of intensely yellow colour	-
8	Saponin	distilled water	persistent foam	-
9	Tannins	drops of ferric chloride lead sub acetate solution	Transient greenish to black color A cream gelatinous precipitation	-

* - absent, + trace, ++ present, +++ abundant

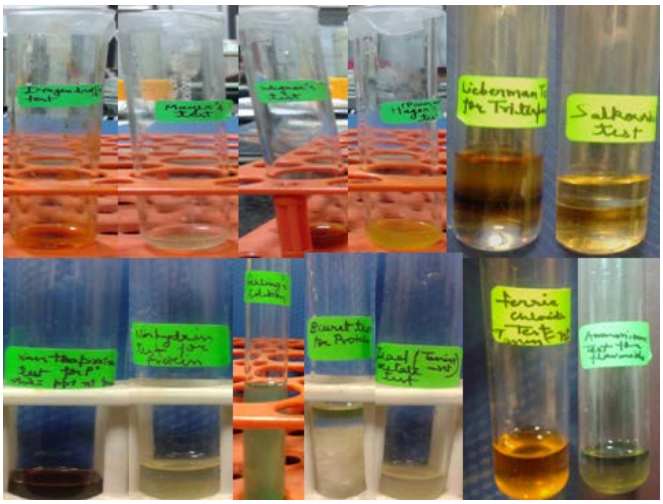

Fig 2 Phytochemical screening of methanolic extract of roots of *achyranthes aspera linn.*

Figure 3: TLC identification of terpenoids from methanolic root extract of achyranthes aspera after derivatization.

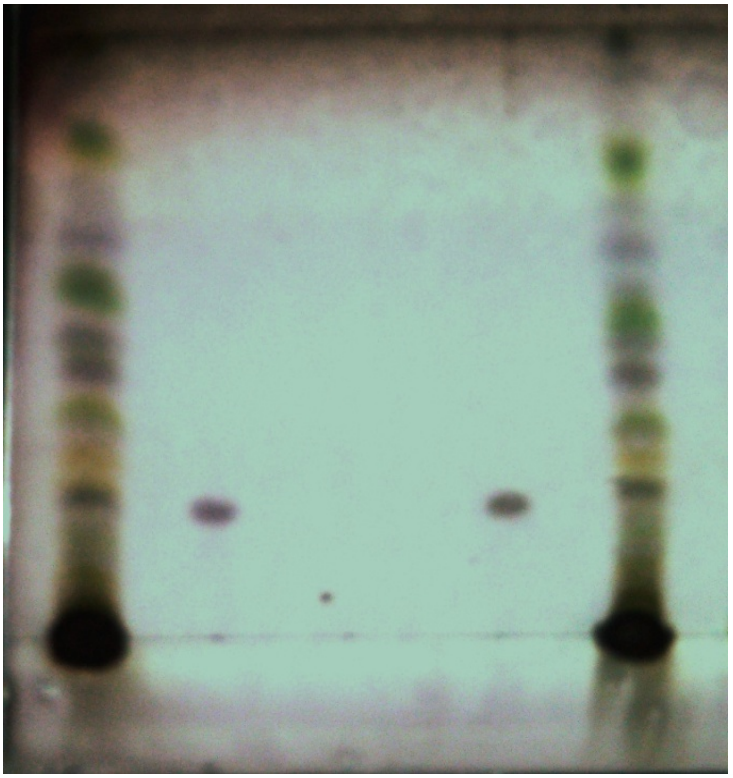

Table 2: Results of TLC of methanolic extract of achyranthes aspera linn.:-
Stationary Phase: Silica gel. 60-120 mesh size (Merk).

Sample	Solvent system(7.8:2.2:0.75 ml)	No. of spot	Rf Values
Root extract	Toluene:Methanol:Formic acid	7	0.19,0.20,0.45,0.5,0.58,0.66,0.83
Standard1(Oleanolic acid)	Toluene:Methanol:Formic acid	1	0.19
Standard2(Ursolic acid)	Toluene:Methanol:Formic acid	1	0.20

IV. CONCLUSION

The selected Ethanomedicinal plant is source of secondary metabolitesi.e.alkaloids,Flavanoids,terpenoids,steroids,glycosides and reducing sugars.Medicinal plants play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. This phytochemical screening study established a significant scope to develop a broad spectrum use of roots of *Achyranthes aspera* in herbal medicine and as a base for the development of novel potent drugs and phytomedicine.

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AUTHORS

First Author – Asmita Singh ,M.Sc,Department of Botany, University of Rajasthan, Jaipur, Rajasthan
Email:-asmitaivri@gmail.com
SecondAutho–ManishaMathur,P.hD ,Lecturer ,R.Rcollege,Alwar
Third Author –Ajay Kumar, Scientist, Indian veterinary Research Institute ,Bareilly
Correspondence Author –
Asmita Singh ,M.Sc ,Department of Botany, University of Rajasthan, Jaipur, Rajasthan