Int J Pharm Bio Sci 2012 July; 3(3): (B) 1054 - 1062

Research Article Bio Chemistry

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

PHYTOCHEMICAL STUDIES ON ELYTRARIA ACAULIS

N. KIRUTHIKA, R.DHIVYA, K.KALAISELVI*, P.KANIMOZHI AND K.PANNEERSELVAM.

Department of Biochemistry, Vivekanandha college of Arts and Science for women, Tiruchengodu, Namakkal (dt), Tamilnadu.

ABSTRACT

The plant Elytraria Acaulis belonging to the family Acanthaceae. The plant mostly found in hills. Stem less perennial herb with 1-several unbranched flowering stems up to c-30CM tall. Leaves in a basal rosettle, subsessile, elliptic to obovate, up to 18cm long, hairy, particularly on the veins. Flowers in 1-several spikes held in tight apiculate, overlapping bracts. Bracts and flowering stem bluish green. Corolla white, lower lip and lateral lobes spreading, 2- Lobed flowers often not opening capsule 5.5-6.5mm long hairless. The phytochemical screening of methanol extract shows the presence of chemical compound like Alkaloids, Flavonoids, Protein, Amino Acid, Glycosides, Carbohydrates, Phenol, Steroids, Saponins and Tannins. The chromatographic studies give various spots (Hptlc)with methanol extract may confirm the presence of alkaloidal contents in the plant.

KEY WORDS: **Elytraria Acaulis, Methanol extract,Hptlc.**

Corresponding author

K.KALAISELVI

Department of Biochemistry, Vivekanandha college of Arts and Science for women, Tiruchengodu, Namakkal (dt), Tamilnadu.

INTRODUCTION

Plants are the unending source for a number of compound which can maintain the health of human being plant have been the corner stone of pharmacy not only in ancient times but also in the area of modern drug discovery. The medicinal plant that possesses therapeutic properties (or) beneficial pharmacological effect on the animal body generally designated as “medicinal plant” all human being require a number of complex organic and inorganic compounds in diet to meet the need for their activities. The important constituents of diet are carbohydrates, fats, protein, and vitamin, minerals and water. India is called the botanical garden of the world for its natural resources. Over 6000 plants in India are identified which are used in traditional, folklore and herbal medicine. Among 1500 medicinal plants, 500 are commonly used.

Plants have been the most important for human health. Many modern medicines are derived from plant either extracted from plant themselves or artificially synthesized to copy plant chemical compounds. WHO (world health organization) estimates that 80% of the world population relies on plants for their primary health care. Elytraria Acaulis is widely distributed in South Africa and India. The family Acanthaceae consists of several important medicinal plants with wide range of biological activities and interesting phytochemical constituents. Elytraria Acaulis traditionally used in the treatment of asthma, migraine, leucorrhoea, snake bite. It is also used to treat antihypoglycemic activity.

The various forms of chromatography on differential solubility or absorption of compounds to separate molecules between a stationary phase and a mobile phase.So it is no wonder that it is difficult to pin down the exact beginning of the technique. The modern analytical tool HPTLC is a powerful and quantitative analytical task. HPTLC is playing an important role in today's analytical world, but not in competition to HPLC but as a complementary method. This describes HPTLC features and basic steps involved in instrumentations.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

The fresh leaves of Elytraria Acaulis were collected in area of pesticides and other contaminants from the surrounding in hills in Namakkal. The plant material which includes fresh green leaves which were washed, shade and dried at room temperature for 15 days.

SAMPLE PREPARATION

The leaves were washed, and cut into small pieces and dried for five days. The sample were ground into powder and stored each in an air tight bottle prior to use for analysis. The powdered plant leaves were used for the extraction procedure. Extract about 50g of the plant sample with methanol.

DETECTION OF CARBOHYDRATE

MOLICSH’S TEST

The extracts were treated with 2-3 drops of 1% alcoholic ALPA napthal and 2ml of concentrated sulphuric acid was added along the sides of the tube. The formation purple ring between two layers shows the presence of Carbohydrates.

FEHLING’S TEST

The extract were treated with Fehling’s A and B solution and heated for few minutes. Formations of brick red precipitate shows the presence of reducing sugar.

BENEDICT’S TEST

The extracts were treated with Benedict’s reagent and heated for few minutes. Formations of red precipitate show the presence of reducing sugar.

DETECTION OF GLYCOSIDES

LEGAL'S TEST

To the hydrolyzed 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide. The pink colour changes into red shows the presence of glycosides.

BORNTRAGER'S TEST

Hydrolyzed was treated with chloroform and was added. The pink colour changes in to red showed the presences of Glycosides.

DETECTION OF PROTEIN AND AMINO ACID

MILLON'S TEST

The extracts were treated with millons's reagent. The precipitate was formed with the extract, which shows the presence of Proteins.

NINHYDRIN'S TEST

The extracts were treated with Ninhydrin's reagent. The purple colour was formed with extract, which shows the presences of Protein.

BIURET'S TEST

To the extract, equal volume of 5%Sodium Hydroxide solution and 1% Copper Sulphate solution was added. A violet colour formation indicates presence of Amino Acids.

DETECTION OF FIXED OILS AND FATS

SPOT TEST

Small quantities of extract were placed between two filter papers. The productions of stain with alcoholic extracts show the presence of Fats and Fixed oils in the extract.

SAPONIFICATION TEST

Few drops of 0.5N Alcoholic Potassium Hydroxide was added to the extract with few drops on Phenolphthalein solution. Later, the mixture was heated on water both for 1-2hrs. The soap formation indicates the presence of Fat and Fixed oils in the alcoholic extract.

DETECTION OF GUMS AND MUCILAGE'S

RUTHENIUM RED TEST

A small quantity of extract was suspended with water and added Ruthenium red solution. Formation of pink colour showed the presences of Gums and Mucilage's.

DETECTION OF ALKALOIDS

A small quantity of the extract was treated with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with Alkaloid reagent such as

a) Mayer's reagent (cream precipitate)
b) Dragendorff's reagent (reddish brown precipitate)
c) Hager's reagent (yellow precipitate)
d) Wagner's reagent (reddish brown precipitate)

DETECTION OF FLAVONOIDS

1. A small quantity of the extract was dissolved in alcohol to that Magnesium metal and concentrated Hydrochloride Acid was added. Colour change shown the presence of Flavonoids.
2. Small quantities of the extracts were treated with Sodium Hydroxide solution. Formation of yellow colour indicates the presence of Flavonoids.
3. Take small quantities of alcoholic extracts, heated on a water both after acidification for 15 min. Then extracted with Chloroform, to the Chloroform layer add new pieces of zinc granules followed by a drop of concentrated HCL

DETECTION OF PHYTOSTEROLS

LIEBERMAN BURCHARD TEST

The above prepared chloroform solution was treated with few drop of concentrated sulpuric acid. A bluish green colour solution obtained in chloroform extract shows the presences of Phytosterols.

SALKOWSKI TEST

To test 1ml of above prepared chloroform solution few drop of concentrated sulpuric acid were added. Formation of brown ring with chloroform extract indicates the presences of Phytosterols.

DETECTION OF TANNINS- PHENOLIC COMPOUND

FERRIC CHLORIDE TEST

To the filtrates few drop ferric chloride was added. (violet colour precipitate)

LEAD ACETATE TEST

To the filtrates few drop of lead acetate solution was Added.

GELATIN TEST

To the extract, 1ml of 1% solution was added.

DETECTION OF SAPONINS

FOAM TEST

The extract were diluted with 20 ml of diluted water and then agitated in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicates the presence of Saponins.

HAEMOLYSIS TEST

About 2ml of blood was taken in two test tubes separately. To one of the tubes, equal quantity of water was added. To the other test tube an equal quantity of methanolic extract dissolved in water was added. A clear red liquid was formed in the first test tube, which indicates that red blood corpuscles were Haemolysis. The extract in the second test tube also Haemolysis. It indicates the presence of Saponins

DETECTION OF STEROID

To the methanol extract add few drop of acetic anhydride and a drop of concentrated sulphuric acid. Appearance of green or brown colour was the end point.

HIGH PERFORMANCES THIN LAYER CHROMATOGRAPHY (HPTLC)

Methanol plant extract 100mg was dissolved in 1ml Methanol and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis. 2µl of above test solution and 2µl of standard solution was loaded as 5mm band length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The sample plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Alkaloid) and the plate was developed in the respective mobile phase up to 90mm.

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254nm and UV366nm.The developed plate was sprayed with respective spray reagent (Alkaloid) and dried at 100° C in Hot air oven. The plate was photo-documented in Day light mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

PHYTOCHEMICAL STUDY
QUALITATIVE PHYTOCHEMICAL ANALYSIS OF ELYTRARIA ACAULIS METHANOL EXTRACT

Plant Constituents	methanol extract of Elytraria Acaulis
Carbohydrate	+
Glycosides	+
Protein and amino acids	+
Alkaloids	+
Flavonoids	+
Phytosterols	+
Tannins and Phenolic	+
Saponins	+
Steroids	+

(+) = Present

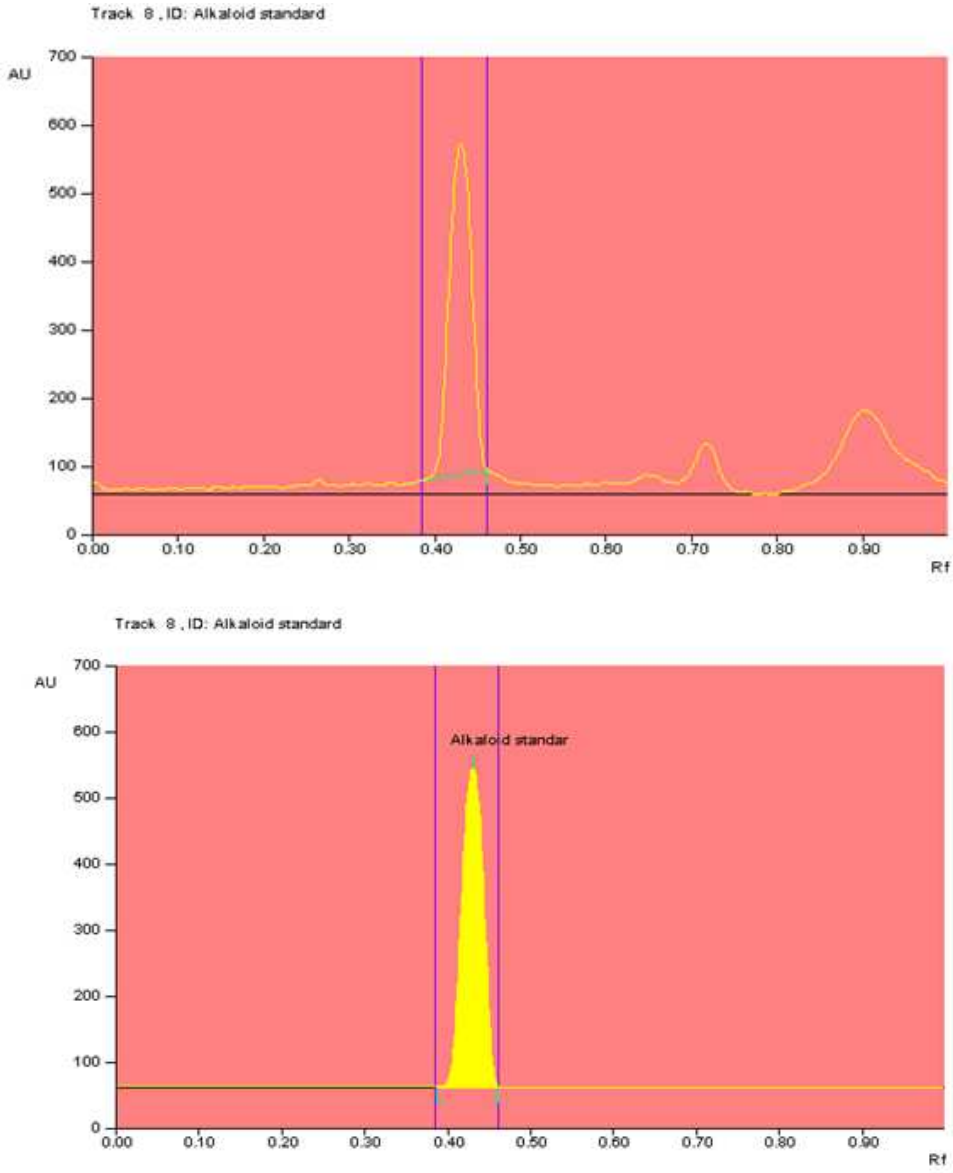
HPTLC REPORTS OF METHANOL EXTRACT OF POWDERED PLANT OF ELYTRARIA ACAULIS

HPTLC Datas
Mobile phase: Ethyl acetate-Methanol-Water (10 : 1.35 : 1)
Detection wave length =254nm

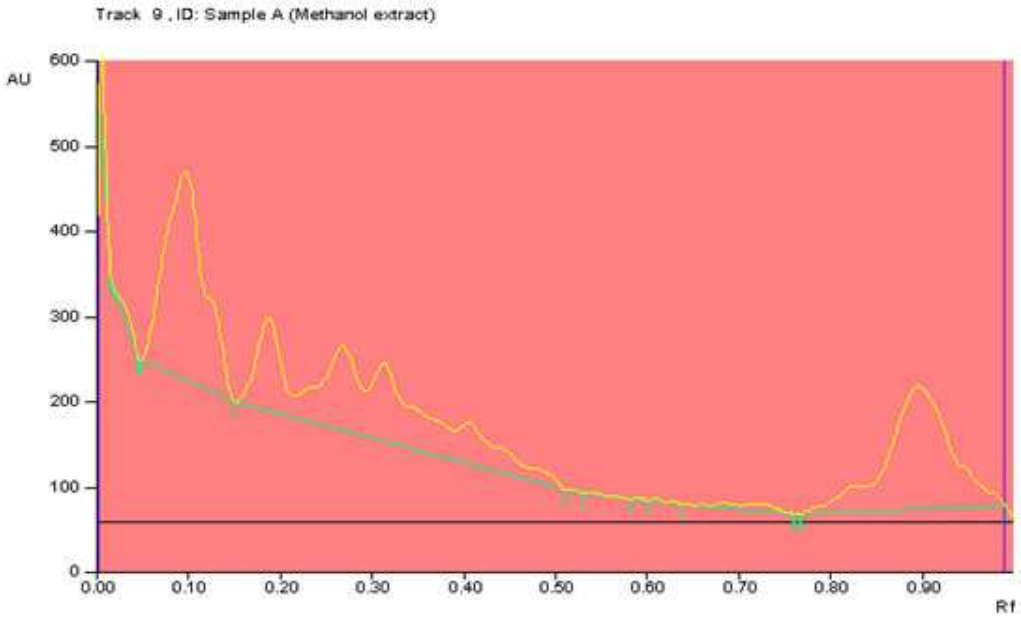
PEAK TABLE

Track	Peak	Rf	Height	Area	Assigned Substance
STD	1	0.43	511.3	13919.7	Alkaloid standard
Sample A	1	0.04	16.6	194.8	Unknown
Sample A	2	0.10	245.0	9805.2	Alkaloid 1
Sample A	3	0.19	109.1	2709.8	Alkaloid2
Sample A	4	0.27	100.1	3145.8	Alkaloid
Sample A	5	0.31	92.8	4270.1	Unknown
Sample A	6	0.41	49.6	1389.6	Alkaloid
Sample A	7	0.44	29.5	647.5	Unknown
Sample A	8	0.48	16.6	372.1	Unknown
Sample A	9	0.83	31.0	733.2	Unknown
Sample A	10	0.89	146.5	8630.2	Unknown

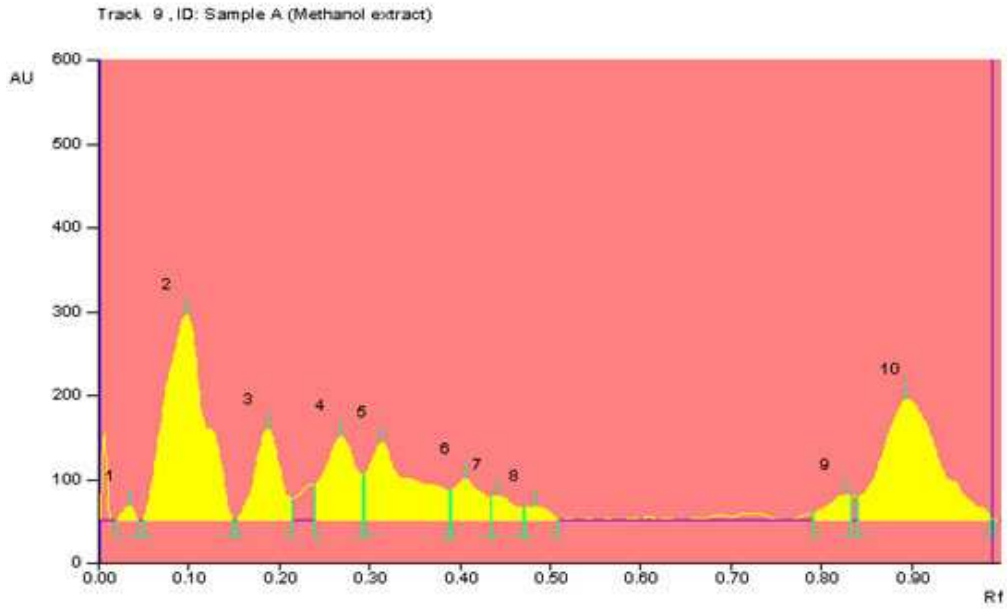
STD- ALKALOID STANDARD BASELINE DISPLAY (SCANNED AT 254nm)
TRACK STD – ALKALOID STANDARD PEAK DENSITOGRUM DISPLAY (SCANNEDAT254nm)



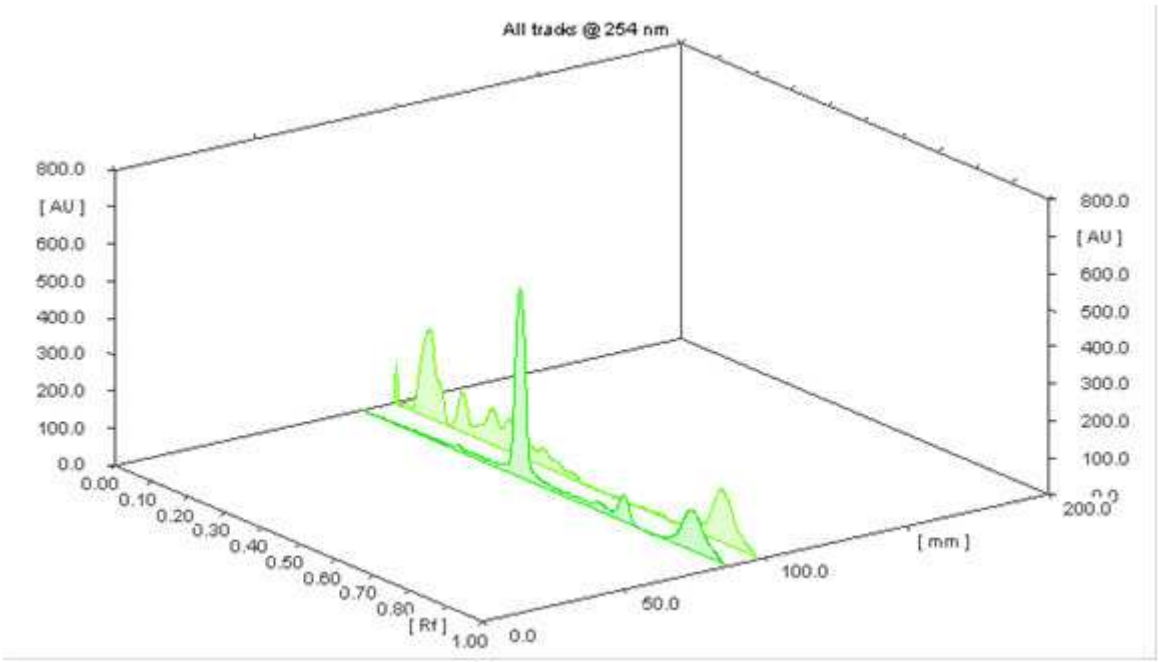
TRACK A- SAMPLE A METANOL EXTRACT PLNT SAMPLE DISPLAY (SCANNED AT 254nm)



TRACK A- SAMPLE A METHANOL EXTRACT PLANT SAMPLE PEAK DENSITOGRAM DISPLAY (SCANNED AT 254nm)



3D DISPLAY OF ALL TRACK



RESULT AND DISCUSSION

The plant Elytraria Acaulis belongs to the family Acanthaceae. The phytochemical screening of methanol extract shows the presence of chemical compounds like alkaloids, Flavonoids, carbohydrate, glycosides, protein, tannin, amino acid, Saponins, phenol. The maximum absorbance of the methanol extract was also studied.

Since the chromatographical studies shown various spots (HPTLC) with the methanol extract may confirms that presence of different alkaloids content in the plant. The HPTLC were done. The methanol extracts were subjected to HPTLC procedure. The methanol extract of plant shows spots with respect to the detection wave length of 254nm and 366nm respectively.

The plant Elytraria Acaulis (Acanthaceae family) is claimed to have medicinal uses such as asthma. Migraine, leuwrrhoea, snake bite and also used to treat the hypoglycemic activity.

REFERENCE

1) Harish Chandra andola. High performance thin layer chromatography (HPTLC): A modern analytical tool for biological analysis .Natural science 2010.8(10): 58-61.
2) Kabmarawa, D., Khan, M.E., Punah, A.Mand Hassan. M (2008), phytochemical screening of extract from Khaya senagalensis against human pathogenic bacteria*, African Journal of Biotechnology 2008; 7(24), 4563-4566.*
3) Khushboo, P.S., Jadhav,VM and Kadam, V.J (2009) Development and validation of a HPTLCmethod for determination of psoralea lorylifolia (Bavachi), *International Journal of Pharmtech Research, 1(4),1122-1128.*
4) Pandey CN, Raval BR, Malis, Salvi H. 2005. MEDICINAL PLANT OF Gujarat (compilated) Gujarat ecological education and research (GEER) foundation, Gandhinagar.

5) Olsen ; C Sand H.O Larsen,Alpine medicinal plant trade and Himalayan maintain livelihood strategies.geo,169,243-254 (2003)
6) Patel M.R Patel, K.K. Bhatt and B.G Patel, “HPTLC method development and validation: quantification of paliperidone in formulation and in vitro release study”, analytical method vol2, no 5, PP, 525-531, 2010.
7) Sofowora, A. 2002 medicinal plant and traditional medicine, WHO,
8) Sachin Rakesh,U,Salunkhe,V.R.,Dhabale,P.N and Burade,K.B (2009) HPTLC method for quantitative determination of gallicacid in hydroalcoholic extract of dried powder of nymphaeamstellate wild, Asian Journal of chem..,(2),131-134.