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Evaluation of phytonutrients and thinlayer chromatography profiling of sequential extracts of *Andrographis Echioides* Nees

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ABSTRACT

Andrographis echioides Nees is a herbaceous plant, belonging to the family acanthaceae has been used for the treatment of fever, asthma and liver diseases. The objective of the study was to evaluate the phytonutrients in stem, root and leaf of A.echioides and thinlayer chromatography (TLC) profiling of sequential extracts of A.echioides. Proximate analysis includes moisture, ash, carbohydrate, protein and lipid content were determined. Vitamins (thiamine, riboflavin ascorbic acid and alpha tocopherol) and minerals (iron, calcium and phosphorus) were also analyzed. The leaf extract of A.echioides were prepared using different solvents which includes hexane, chloroform, acetone, ethylacetate, methanol and water. TLC profiling of all the extracts was studied. Among the various parts of A.echioides, leaves possess maximum amount of proteins, lipids and carbohydrates. Significant amount of iron, calcium and phosphorus was found to be present in A.echioides.Calcium was found to be maximum in root, stem and leaf (31.2, 61.2 & 74.8 mg/g) of A.echioides. Stem and leaf part of the plant contains higher amount of non enzymic antioxidants which includes thiamine, riboflavin, tocopherol and ascorbic acid. Relative front values of bands observed for the sequential extracts lies between 0.12 to 0.75 confirms the presence of phytochemicals which includes flavonoids, saponins, terpenoids, phenols and aminoacids.TLC profiling of all the extracts of A.echioides confirmed the presence of number of phytochemicals and different Rf values of phytochemical compound reflects an idea about their polarity. The results obtained in the present study indicated the whole plant, Andrographis echioides Nees as a rich source of phytonutrients, phytochemicals and minerals that contribute to its effectiveness as a traditional medicine.

Keywords: Phytonutrient, Phytochemical, *Andrographis echioides*, Thin layer chromatography

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INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Herbalism is a traditional medicine or folk medicine practice based on the use of plants and plant extracts[1].Many of the herbs and spices used by humans to season food yield and useful medicinal compounds [2-3].Herbal therapy is used to treat a large variety of ailments and symptoms ,eg, inflammation, fever and pain; however, there are no adequate experimental evidences about their effectiveness[4-5].

The plant *Andrographis echioides Nees* is an annual herb, distributed throughout India and Srilanka. It belongs to the family Acanthaceae.The herb is useful in the treatment of dysentery, diabetes, swelling and also used for liver disease and jaundice[6]. Vernacular names of *A.echioides* in Malayalam (Pitumba),Tamil (Gopuram thangi,

Peythumbai) and in Telugu (Chalavala purikada). Plants belonging to the genus Andrographis have been shown to contain, a diterpenoid lactone and flavonoids. The group of flavonoids is famous for its anti- inflammatory, anti- allergic, anti- thrombotic, vasoprotective and protection of gastric mucosa properties. These properties have been attributed to influence of flavonoids and production of prostaglandins and their antioxidant effects[7].

MATERIALS AND METHODS

Collection of Plant material : *Andrographis echioides Nees* the whole plant were collected from Sunaiyamparai village, Kancheepuram district, Tamilnadu and authenticated by Dr.S.Jayaraman, Director of Plant Anatomy Research Centre, West Tambaram, Chennai (Reg no: PARC/2014/2066).

Preparation of plant extract: Fresh matured plant was cleaned, washed and shade dried for 10 days. After drying it was powdered in a blender and kept in air tight container. 50gm of dried plant powder was taken and soaked in 200 ml of water, methanol, ethyl acetate, acetone, chloroform and hexane solvent. It was kept in orbital shaker for 24 hours at 37ºC. The extract was filtered using whatmann no. 1 filter paper. Extracted solvent were allowed for evaporation in a drier.

QUALITATIVE ANALYSIS OF PHYTONUTRIENTS

Moisture Content: Moisture content of the plant (root, stem and leaf) was estimated by drying a known biomass of the plant in hot air oven at 70 ± 2ºC to constant weight. This was cooled in dessicator and weighed. The loss in weight was expressed as percentage of moisture[8].

Ash Content : A known weight of the plant material was initially charred on red silica crucible and placed in a muffle furnace at 400 - 450ºC for 6 h till the charred material became white. The crucible was allowed to cool to room temperature in a dessicator and reweighed. The difference in weight was expressed as total ash content [8].

Estimation of Carbohydrate: Carbohydrate was estimated by Dubois method [9]. To 50 mg of plant sample, 2.0 ml of 5% trichloro aceticacid was added and centrifuged at 2000 rpm for 10 minutes. To the supernatant add 100 ml of 5% ethanol and kept overnight in cold condition. Again the tubes were allowed to centrifuge at 4000 rpm for 10 minutes. To the dried precipitate, 2 ml of 1N sodium hydroxide was added. To the extracted sample 1ml of distilled water, 1ml of 5% phenol followed by the addition of 5ml of concentrated sulphuric acid and allowed to incubate at room temperature for 10 minutes. Optical density was read at 490 nm using spectro photometer.

Estimation of protein: Protein was estimated by Lowry's method [10].Different dilution of bovine serum albumin (BSA) solutions are prepared by mixing stock BSA solution (1mg/ml) with water. The BSA concentration range is 0.05 to 1mg/ml. From these different dilutions, pipette out 0.02ml protein solution to different test tubes and add 2ml of alkaline copper reagent. This solution is incubated at room temperature for 10 minutes. Then add 0.2 ml of Folin-ciocalteau reagent solution to each tubes and incubate for 30 minutes. Adjust the colorimeter with blank and the OD was measured at 660nm.

Estimation of lipid: Lipid was estimated by Bligh and dyer method [11].

Estimation of pigments: Chlorophyll a ,b and total carotenoid was determined by Lichenthaler method [12].

Estimation of vitamin : Tocopherol and ascorbic acid are determined by colorimetric method [13][14], vitamin thiamin and riboflavin are determined by spectro fluorimetric method.

Estimation of minerals: Minerals which includes iron, calcium and phosphorus were determined from plant ash. Iron was determined by Ramsay method[15], calcium and phosphorus are determined by Titrimetric and Fiske subbarow method[16].

BIOACTIVE COMPOUND DETECTION USING THIN LAYER CHROMATOGRAPHY

TLC was carried out to isolate the principle components that were present in extract of the plant. TLC studies were carried out for six solvent extracts which includes water (AqEAE), methanol (MEAE), ethyl acetate (EtEAE), acetone(AEAE), chloroform (CEAE) and hexane (HEAE) on commercially available precoated TLC sheet SIL

G/UV254 (Machery- Nagel). The different solvent system of different polarities are prepared and TLC studies are carried out to select the solvent system capable of showing better resolution.

Solvent phase: The different solvent system used were Toluene: ethylacetate: Diethylamine, for terpene (Toluene: chloroform: ethanol) and Flavonoid(Toluene: Ethyl acetate) .

Methods: The extract were applied on precoated TLC plates using capillary tubes and developed in a TLC chamber using suitable mobile phase. The developed TLC Plate were air dried and observed under ultra violet light at both 254nm and 366nm.They were sprayed with spraying agent and some were placed in iodine chamber for development of color as separated bands. The movement of analyte was expressed by its retention factor (R_f) values were calculated for different sample.

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}$$

RESULTS AND DISCUSSION

Estimation of carbohydrate, total protein and Lipid content

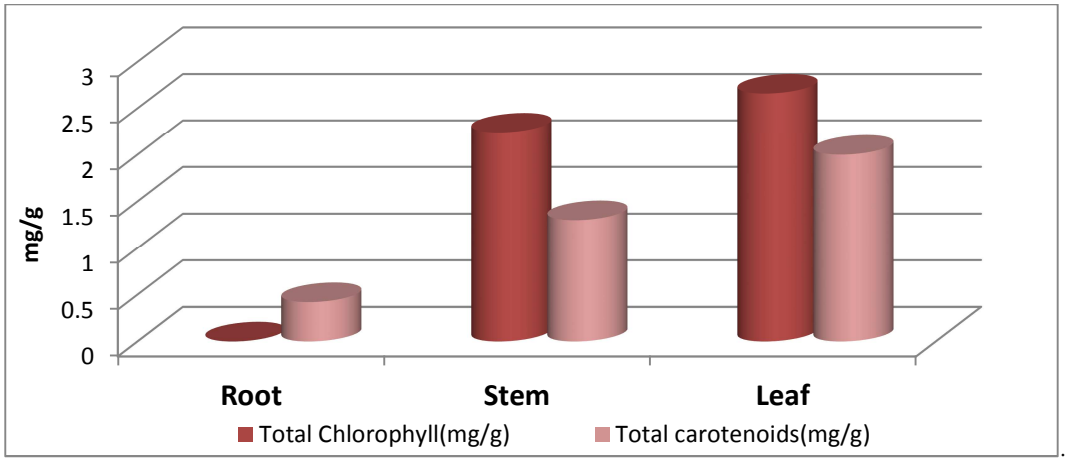
Proximate composition of root, stem and leaf of *A.echioides* which includes carbohydrate , protein, lipid, moisture and ash content was depicted in **Table 1**. The moisture content of the root, stem and leaf were17.6, 9.5 and 11% respectively.The ash content of the root, stem and leaf of *A.echioides* was 81.3, 89.6 and 90.3% respectively. The ash content of the leaves was higher than stem and roots of *A.echioides*, whereas the root retains increased moisture content. Total protein content was found to be higher in all the parts of the plant than lipids and carbohydrates. Among the various parts leaves possess maximum amount of protein, lipid and carbohydrate (101.3, 8.36 and 5.0 mg/g) respectively. This suggests that plant is a good dietary source of the proximate principles.

Table 1: Proximate composition of root, stem and leaf of *Andrographis echioides Nees*

S.no	BIOCHEMICALS	Root	Stem	Leaf
1	Moisture content(%)	17.6±0.84	9.5±0.5	11.2±0.81
2	Ash content(%)	81.3±2.8	89.6±2.4	90.3±2.5
3	Carbohydrate (mg/g)	0.71±0.08	1.56±0.08	5.4±0.26
4	Protein(mg/g)	76±3.2	40.4±1.73	101.3±3.4
5	Lipid(%)	4.0±0.16	5.86±0.24	8.36±0.54

Values were expressed in mean ± SD (n=3 determination)

Figure-1 Pigments of *Andrographis echioides*



Values were expressed in mean ± SD (n=3 determination)

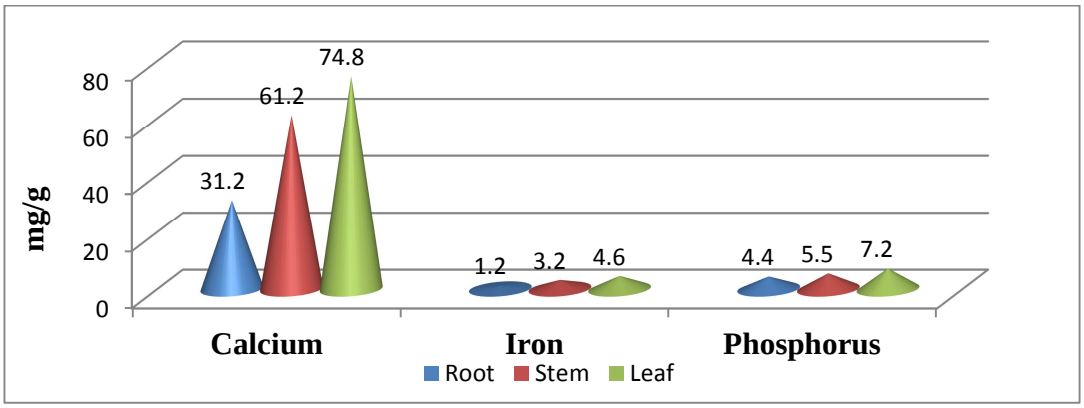
Estimation of Pigments

Pigment composition of *A.echioides* was depicted in **Figure 1.** Total chlorophyll and carotenoid in leaves of *A.echioide*s was found to be 2.66 and 2.0 mg/g respectively. Total chlorophyll content was found to be greater compared to total carotenoid. In plants and algae carotenoids have both photosynthetic and photo protective role, reported that naturally occurring carotenoids other than β- carotene have exhibited anticancer activity are being concluded further as potential chemo preventive agent[17-19].

Estimation of Vitamins and Minerals

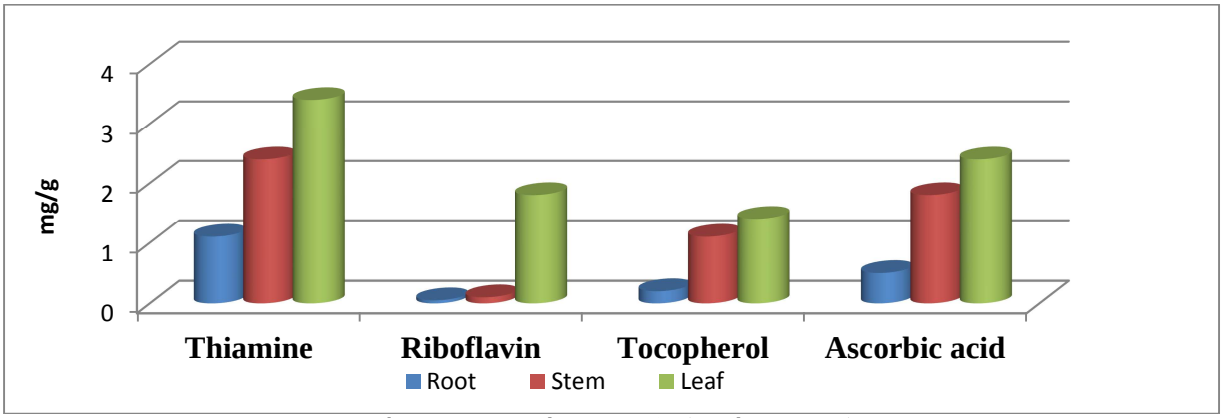
Significant amount of Iron, Calcium and Phosphorous is found to be present in *A.echioides* (**Figure -2**). Among the minerals quantified calcium was found to be maximum in the root, stem and leaf of. *A.echioides.* These minerals are essential for many enzyme system in carrying out biochemical function like energy production, protein metabolism and bone formation etc.

Figure 2: Analysis of minerals of *Andrographis echioides Nees*



Values were expressed in mean ± SD (n=3 determination)

Figure 3: Analysis of vitamins in the whole plant of *Andrographis echioidesNees*



Values were expressed in mean ± SD (n=3 determination)

Figure 3 depicts the levels of vitamins. Thiamine was found to be present in greater amount in root(0.86),stem(2.4) and leaves(3.4mg/g) of *A.echioides* when compared to riboflavin, tocopherol and ascorbic acid(non enzymic antioxidants). *A.echioides* leaves possess higher amounts of vitamins when compared to stem and root. Vitamin riboflavin, tocopherol and ascorbic acid has been determined to the extent of 1.8, 1.4 and 2.4 mg / g of leaves of *A.echioides* .Antioxidants possess diverse biological activities such as anti inflammatory, anti artherosclerotic and anti carcinogenic activities.

Profiling of sequential extracts of *Andrographis echioides*

TLC Profiling of methanol, ethyl acetate and acetone extracts of *Andrographis echioides* was depicted in figure 4A using solvents Toluene:ethylacetate: Diethylamine (7:2:1).TLC profile of the extracts showed more than 5spots respectively with β carotene as standard.TLC profile of hexane, methanol and aqueous extracts of *A.echioides* in solvent system Toluene :Chloroform: Ethanol (4.5:4.5:1) (Figure 4B).This solvent system was used for detecting terpenoids,the methanol extract showed more bands.

Figure 4A TLC Profiling of sequential extracts of *A. echioides* (Toluene:Ethylacetate:Diethylamine)7:2:1 After exposure to UV light

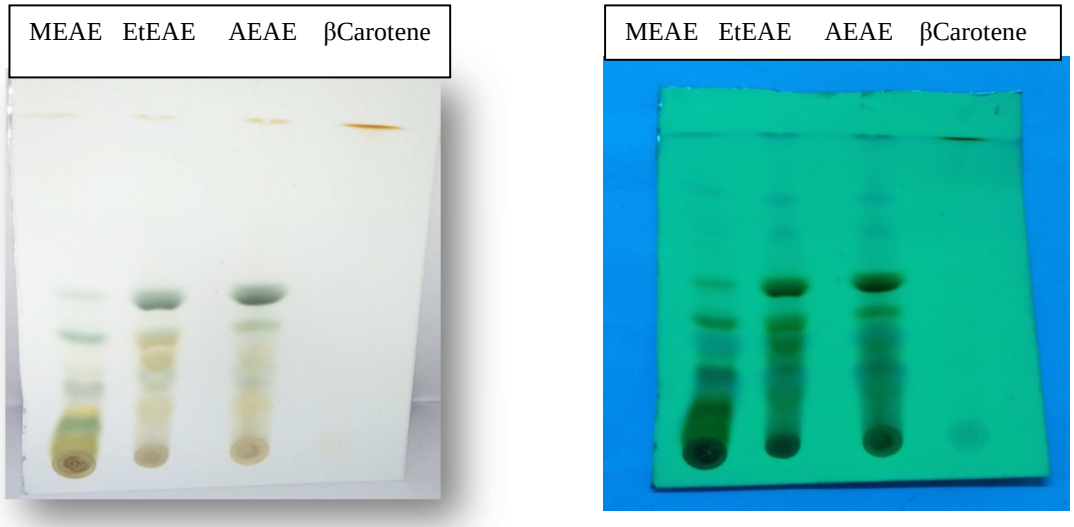
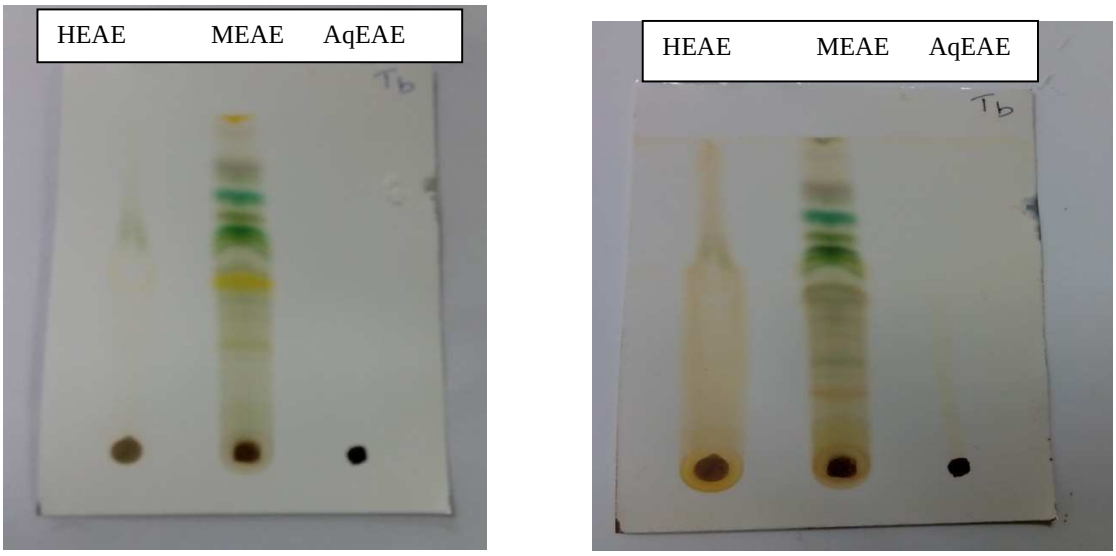
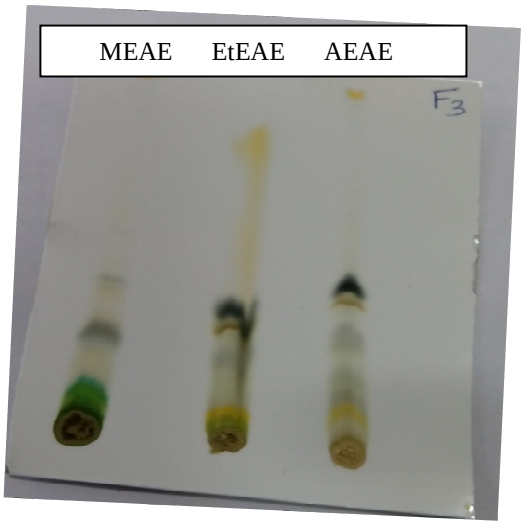


Figure 4 B Terpene (Toluene; Chloroform: Ethanol) After exposure to Iodine vapour





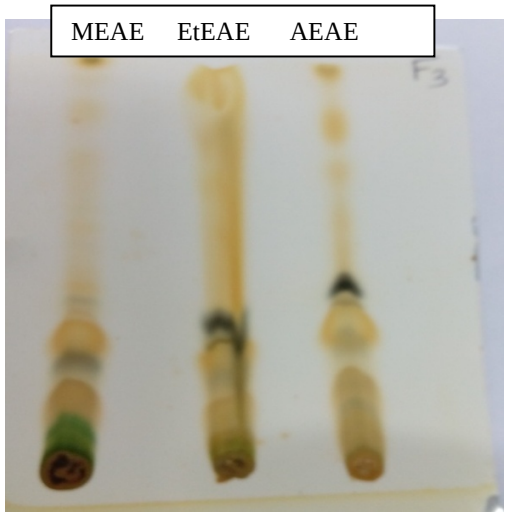


Figure 4C Flavonoid (Toluene : ethylacetate) **After exposure to Iodine vapour**

Table 2- Rf values of phytochemicals of sequential extracts of *Andrographis echioides*

S.no	Phytochemicals	Rf Value
1	Flavonoid	0.46
2	Saponin	0.42,0.61
3	Terpenes	0.28,0.66
4	Phenol	0.44,0.67
5	Aminoacids	0.65

TLC Profiling of methanol, ethyl acetate and acetone extracts of *Andrographis echioides* was depicted in **figure 4C** using solvents system Toluene:ethylacetate (9.5:0.5) for detecting flavonoids.All the extracts showed prominent spots after exposure to iodine.**Table -2** depicts the relative front(Rf) in cm obtained in TLC of the crude extracts of *A.echioides*.TLC profiling of crude extract of *A.echioides* revealed the presence of compounds having Rf value of 0.28,0.42, 0.44, 0.46, 0.61, 0.65 cm indicating the presence of flavonoid, terpenes, saponin ,amino acids and phenol. Govindachari reported the isolation of a new flavones, echioidinin and aflavone glucoside, echioidin from the acetone extracts of *A.echioides*. Nees[20].

CONCLUSION

Andrographis echioides reveals the presence of phytochemicals and phytonutrients. Among the phytochemical and phytonutrient flavonoid, saponin, steroid, total protein and calcium content was found to be maximum in the whole plant. TLC studies gives the idea about the polarity of compound. This result contributes its effectiveness as a traditional medicine so it can be used for the synthesis of drugs and other medicinal products.

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