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Research Article

PHYTOCHEMICAL EVALUATION OF AN AYURVEDIC DRUG *DICHROSTACHYS CINEREA* (L.) WIGHT & ARN

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ABSTRACT:
The traditional plant, *Dichrostachys cinerea* (L.) wight & Arn. Belongs to the family Mimosaceae, is commonly used by tribal of India and South Africa. The plant is claimed to possess more therapeutic medicinal uses such as anti-inflammatory, diuretic, used in rheumatism, urinary calculi and in diarrhoea, which is evident from literature.. So in the present study an attempt has been made for investigation of chemical constituents of the plant by phytochemical method..Phytochemical studies include qualitative phytochemical analysis, fluorescence analysis, elemental analysis and quantitative estimation of therapeutically important phytoconstituents. This study also included high performance thin layer chromatography of the plant with marker compounds β-amyrin and friedelin.Qualitative phytochemical analysis of leaf, stembark and root revealed the presence of terpenoid and steroid, glycosides, tannin, flavonoids and saponin. The result of elemental analysis indicates the presence of heavy metals within the limit as per W.H.O guidelines. Further the total alcoholic extract of leaf, stembark and root were found to contain rich amount of total phenolic compounds, tannins and flavonoids. And also significant amount of Vitamin C and Vitamin E.HPTLC profile of alcoholic extract of leaf, stembark and root showed the presence of β –amyrin, whereas friedelin was found to present only in leaf. All the above phytochemical findings give detailed phytochemical information of the plant for its standardization.The data retrieved from the observations have been formulated into a diagnostic protocol of *Dichrostachys cinerea*.

Key words : *Dichrostachys cinerea*, Ayurvedic Drug , phytochemical

INTRODUCTION

Herbal medicines are extremely effective hence many of the modern orthodox medicine are based on medicinal herbs.. Intense investigation have been carried out in the historical uses of plant and folklore medicine for finding out new drugs for cancer, Aids, arthritis, diabetes and even for the common cold.An indepth study of ethno botanical and ethno pharmacological reviews revealed one such plant drug mentioned in Siddha and Ayurvedic system of medicine and mostly used by the traditional practitioners in India, Srilanka and South Africa is *Dichrostachys cinerea* (L.) Wight & Arn. belonging to the family Mimosaceae.The genus *Dichrostachys* comprises of twelve species distributed in all over tropical world [1]. Among these *Dichrostachys cinerea* is commonly distributed

in all arid zone of India in dry regions. It is called "*Viddathalai*" in Tamil and "*Veeratharu*" in Ayurveda[2][3].The plant has been claimed in literature to cure inflammations and gout. It is used for relieving pain and eye infection by tribal women in TamilNadu, India [4]. The plant is used for headache, tooth pain, stomach infection and snake bite by Zulu, Xhosa, and Sotho traditional healers of South Africa [5]. Thorough scientific literature review has laid due account on the medicinal potential of *Dichrostachys cinerea* besides meagre scientific investigations. This prompted to undertake a phytochemical investigation of the drug to substantiate its traditional uses. This plant is used by tribal people for various ailments. Hence in the present study an attempt was made to carry out detailed phytochemical study for standardisaton of the plant

AIM AND OBJECTIVE OF THE STUDY

In the present study, an attempt has been made to standardize the plant by phytochemical evaluation. As the plant is used in folklore medicines for various ailments, the plant extract has been screened for various phytocopounds to bring Phytochemical support for the traditional claims. The objective of the work is to carry out Phytochemical analysis of the plant .

MATERIALS AND METHOD

Extraction

The root, stembark and leaves of *Dichrostachys cinerea* were collected from forest area near Padappai, Tambaram, Chennai during June, 2004. It was authenticated by Dr.P.Jayaraman, Director, Plant Anatomy Research Centre, (PARC), West Tambaram, Chennai. A voucher specimen of the plant was deposited at the herbarium of PARC, Tambaram, Chennai. The root, stem bark and leaves were shade dried, coarsely powdered 100g of coarsely powdered material (leaf, stembark and root) were soaked separately with petroleumether (400ml) for 48 hrs. The solution was filtered and filtrate was concentrated and last traces of solvent was distilled off under vacuum. The extraction was carried out twice with petroleumether. The marc was then extracted with chloroform, alcohol and water successively by cold percolation as before. The extractive values of successive extraction of root, stembark and leaf were calculated and given in Table-1

Qualitative phytochemical analysis

The petroleumether, chloroform, ethanol and aqueous extracts of leaf, stem bark and root of *D. cinerea* obtained by successive extraction were subjected to qualitative phytochemical analysis in order to identify the nature of chemical constituents

Elemental analysis[6]

Preparation of sample

Accurately weighed 500 mg of the plant powder (leaf, stembark and root) were taken separately in round bottom flask. 5 ml of concentrated nitric acid was added and refluxed

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for one hr in a hot plate at 80º-100ºC. After heating for one hour the contents of flask were treated with additional 5ml of nitric acid followed by 2ml of 30% (v/v) hydrogen peroxide solution and warmed for 10 min till a clear solution was obtained. It was then cooled, filtered through whatmann-42 filter paper, diluted with de-ionized water and made up to 100 ml in volumetric flask and used for analysis.The absorption is proportional to the concentration of the free atoms in the flame. Working standard solutions of metals were prepared from stock standard solution of 1000 ppm (Merck). Blank solution (Nitric acid) was used to autozero the instrument and the calibration was performed. For various elements wavelength and the range of working standard used in AAS were followed The standards were then analyzed and their absorbances were recorded. A graph of absorption against concentration was plotted. From the calibration curve the elemental concentration of the sample solution was calculated and listed in Table 2.

Estimation of Phytoconstituents

Total flavonoids [7].

Aluminium chloride colorimetric method was used for flavonoids determination [7]. 1 ml of sample solution was mixed with 4.5 ml of methanol, 0.1 ml of 10% w/v aluminium chloride and 0.1ml of 1 M Sodium acetate. It remained at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415nm Perkin Elmer-UV-visible spectrometer (USA). The content of flavonoids was expressed in mg/g.

Total tannins

0.5 ml sample solution was taken and it was made up to 1 ml with distilled water and 1 ml of water served as blank. To this 0.5ml Folins phenol reagent (1:2) and 5ml of 35% w/v sodium carbonate were added and kept at room temperature for 5 min. Blue colour was formed. The colour intensity was read at 640nm (Perkin Elmer UV-Visible spectrophotometer) [8]**.** A standard curve was plotted using 10-100 µg of gallic acid in methanol and the tannin content of the sample was determined.

Total phenols

Total phenols were determined by Folin Ciocalteu reagent[9]. To 0.1 ml sample solution or gallic acid (standard phenolic compound) 5ml of Folin Ciocalteu reagent and 4 ml of sodium carbonate were added. The mixtures were allowed to stand for 15 min and the total phenol was determined at 765nm (Perkin Elmer UV-Visible spectrophotometer). The standard curve was plotted using 10-100 µg of gallic acid in methanol and the total phenol content of sample was determined.

Vitamin E

1.5 ml of sample solution, 1.5ml of standard solution and 1.5 ml of water (blank) were taken separately in three stoppered centrifuge tubes. Volume was made up to 3 ml with ethanol in all the tubes. 1.5 ml of xylene was added to all the tubes, mixed well and centrifuged. 1 ml of xylene layer was transferred to other stoppered tube and 1 ml of α, α’ -dipyridyl reagent was added and mixed. 1.5 ml of the mixture was pipetted out and absorbance was measured in a colorimeter at 460nm against blank. 0.33ml ferric chloride solution was added and mixed. After one min the absorbance was measured at 520nm

against the blank[10]. Then the content of vitamin E in test was calculated using the following formula.

Vitamin E in mg/*l* = (reading of unknown at 520 nm – reading at 450 nm x 0.29) x 10
Reading of standard at 520nm x Weight of sample taken

Vitamin C

The vitamin C was estimated by the method[11]. To 1 ml, 0.1ml of DTC was added and incubated at 37ºC for 3 hrs. After incubation, 1.0 ml of 85 % v/v sulphuric acid was added under ice-cold condition and kept at room temperature for 30 min. The absorbance was measured at 540 nm against a blank in a Perkin Elmer-UV-visible spectrophotometer. The standard graph was plotted, the concentration of standard vitamin C ranges from 10-100 µg. By comparing standard graph, the amount of vitamin C in sample was estimated. The results of concentrations of Phytochemical constituents were tabulated in Table -3

High Performance Thin Layer Chromatography (HPTLC) Profile

HPTLC Methodology

Alcoholic extracts of leaf, stembark and root of *Dichrostachys cinerea* were dried and extracted in chloroform. Chloroform soluble extracts were filtered and made upto 5 ml in standard flasks. Chemical markers Friedelin and β- amyrin were dissolved in chloroform. 20µl of sample and 5 µl of marker solutions were applied on Merck Aluminium plate precoated with silicagel 60 F_{254} using Linomat applicator. The plate was developed in Toluene : Ethyl acetate (9:1 v/v). The plate was dipped in Vanillin-sulphuric and heated in hot air oven at 105ºC till coloured spots appeared. The plate was then scanned at 540nm using Tungsten lamp in Camag HPTLC instrument provided with cats 4.05 version software. The UV spectrum scan of the spots were also carried out.

TLC (Thin Layer Chromatography) Methodology

Alcoholic extracts of leaf, stembark and root of *Dichrostachys cinerea* were dried and extracted in chloroform. Chloroform soluble extracts were filtered and made upto 5 ml in standard flasks. Chemical markers Friedelin and β-amyrin were dissolved in chloroform. 20µl of sample and 5 µl of marker solutions were applied on Merck Aluminium plate precoated with silicagel 60 F_{254} using Linomat applicator. The plate was developed in Toluene: Ethyl acetate 9:1 v/v. The plate was dried and visualized in UV 254 nm and 366nm. The plate was then dipped in Vanillin-sulphuric and heated in air oven at 105ºC till coloured spots appeared.

The results of HPTLC are shown in the Table 4 and 5. HPTLC finger print profiles are depicted in Figures 1 and 2. TLC profile is depicted in Figure 3. The UV spectrum for the leaf, stembark and root extracts with different markers are in Figures 4 and 5. The overlain HPTLC finger print is in Figure 6

RESULTS

The extractive values of leaf, stembark and root of *D. cinerea* were carried out by successive extraction with solvents viz. Petroleumether (60^{o}-80^{o}C), benzene, chloroform,

ethyl acetate, ethanol and water. The extractive values of successive extraction are given in Table1. Alcohol soluble extractive values were found to be comparatively higher than water soluble extractives in all parts of the plant. Among the three parts of the plant, leaf was found to show higher extractive values.

The qualitative phytochemical analysis of petroleumether, chloroform, ethyl acetate, ethanol and aqueous extracts of leaf, stembark and root were performed for the presence of phytoconstituents So in the present study the content of physiologically important elements and heavy metals were analysed by Atomic Absorption Spectroscopy and the results are tabulated in Table 2.

Since the qualitative phytochemical analysis of successive extraction of leaf, stembark and root of the plant showed the presence of tannin, phenolic compounds and flavonoids in alcohol extract, their concentrations in respective extracts were studied. Quantitative analysis of phytoconstituents reveals that the plant *D. cinerea* was found to contain higher amount of tannin, flavonoids, total phenols and vitamin C and the results are tabulated in Table3.

HPTLC finger prints of alcoholic extract of leaf, stembark and root of *Dichrostachys cinerea* are shown in figures 1 and 2. The alcoholic extract of leaf showed 15 peaks with Rf values ranging from 0.01 to 0.98. The Rf values for major peaks in leaf, stembark and root were found to be 0.49, 0.50 and 0.50, respectively which approximately coincided with that of standard β-amyrin having a Rf value of 0.5. The Rf value of friedelin was found to be 0.78.

Peak corresponding to the marker compound Rf value 0.49 (β-amyrin) was observed in all the extracts of leaf, stembark and root of *D. cinerea*. Peak corresponding to the marker compound Rf value 0.78 (friedelin) was found only in the leaf extract.

The TLC plate scanned at 366nm showed pink colour spots for test sample and no coloured spot was seen for marker compounds but the plate on derivatization using vanillin-sulphuric acid followed by heating at 105^{o}C showed well defined bluish grey coloured spot. The TLC of alcoholic extract of leaf, stembark and root showed one spot (bluish colour) with Rf value 0.49 similar to that of β-amyrin (0.49). Leaf extract showed spot corresponding to friedelin (0.78). The TLC profile before and after spray reagent are shown in figure3 ,

The results of UV spectrum of the peak (Rf value 0.49) in leaf, stembark and root extract of the plant were super imposed with that of the standards β-amyrin and friedelin and are shown in figures 4 and 5 and overlain densitogram of extracts and markers are shown in figure6

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Table-1 Extractive Values of of *Dichrostachys cinerea*

Solvents	Extractive Value in (% w/w)		
	Leaf	Stem bark	Root
Petroleum ether 60°-80° C	2.5	0.4	0.5
Benzene	2.1	0.6	0.8
Chloroform	1.2	1.5	1.2
Ethyl acetate	1.0	0.9	1.5
Ethanol	3.5	2.2	2.6
Water	1.5	2.1	3.5

Table-2 Elemental analysis of *Dichrostachys cinerea*

Element	Leaf	Stembark	Root
Iron (ppm)	1.77	1.73	1.98
Copper (ppm)	0.268	0.280	114.8
Zinc (ppm)	0.269	0.265	2.8
Manganese (ppm)	0.589	0.643	162.8
Cobalt (ppb)	193.2	202.2	114.8
Lead (ppm)	0.006	0.005	0.008
Mercury (ppb)	22.60	20.41	16.37
Arsenic (ppb)	0.025	0.027	0.016

Table-3 Quantitative Estimation of Phytoconstituents in *Dichrostachys cinerea*

Phytoconstituents	Amount in mg %		
	Leaf	Stem bark	Root
Total phenols	484.87	279.62	320.47
Total tannins	121.33	62.29	80.90
Total flavonoids	107.99	24.42	35.70
Vitamin C	75.27	50.39	54.04
Vitamin E	89.07	100.4	199.8

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Figure 1. HPTLC FINGERPRINT OF EXTRACTS

LEAF **STEM BARK,** **ROOT**

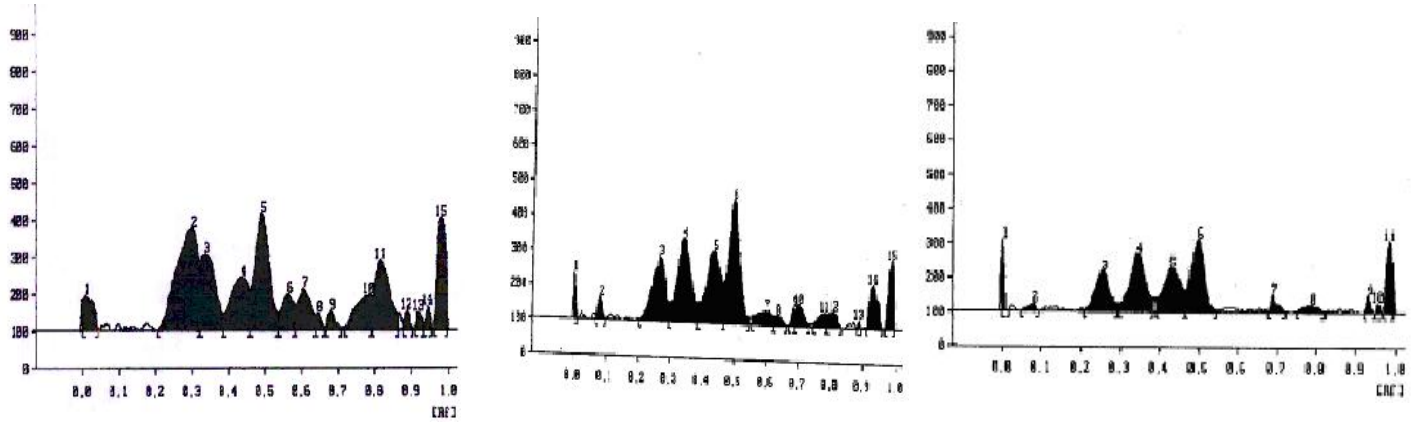


Figure 2. HPTLC FINGERPRINT OF MARKERS

B-AMYRIN **FRIEDELIN**

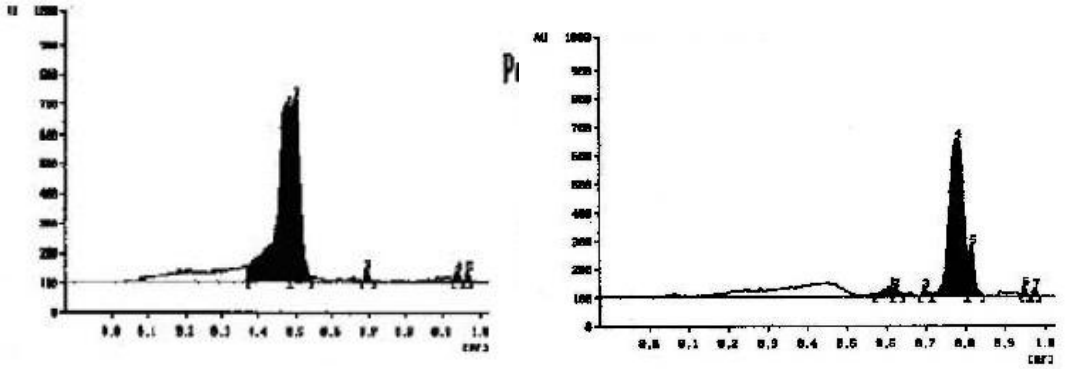
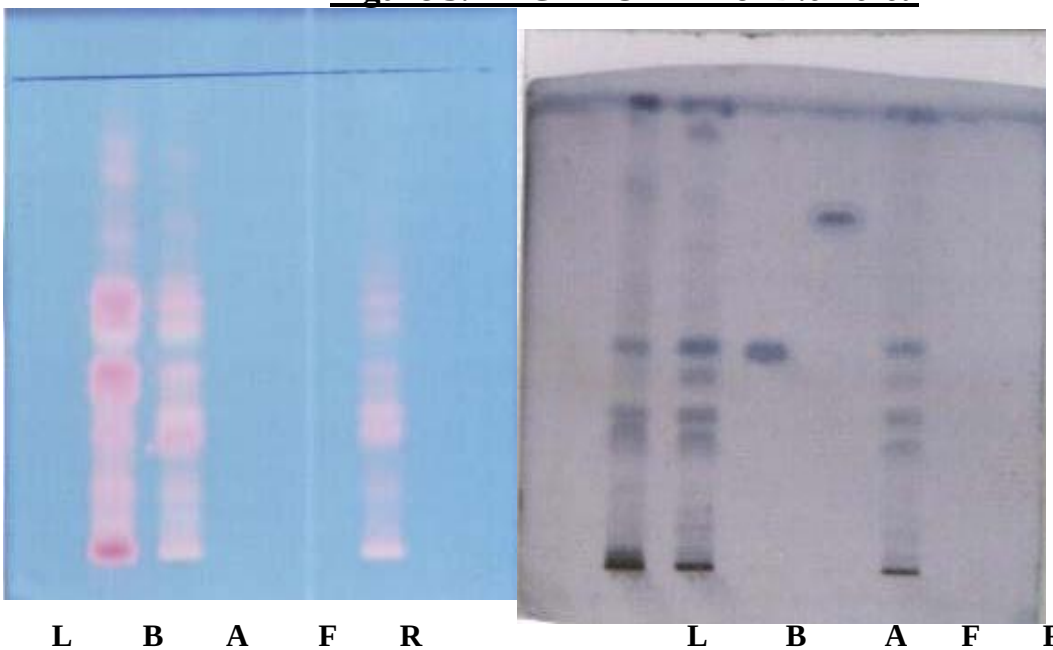


Figure 3. TLC PROFILE of *D.cinerea*

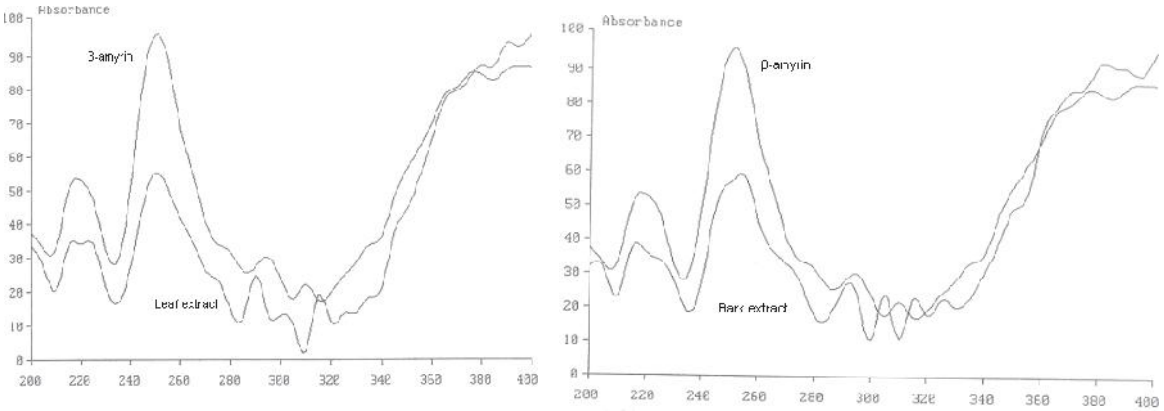


L B A F R L B A F R

λ - 366 nm With Vanillin-Sulphuric Acid

L - Leaf, B – Bark, A – β-amyrin, F – Friedelin, R - Root

Figure 4. SUPERIMPOSED UV SPECTRUM OF EXTRACTS AND β-AMYRIN (MARKER)



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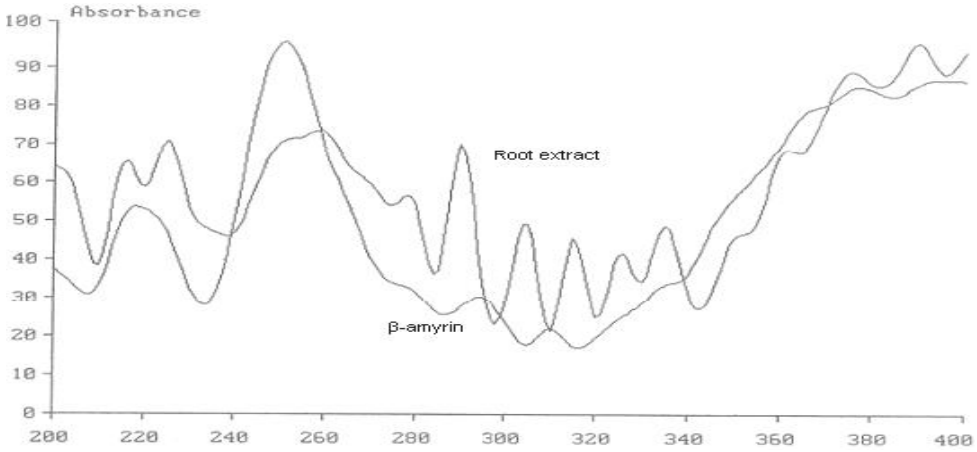
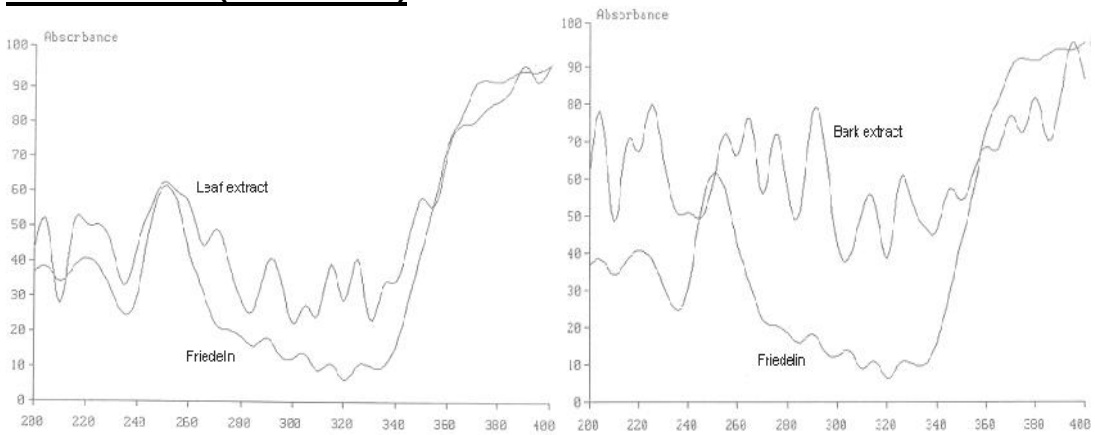


Figure 5. SUPERIMPOSED UV SPECTRUM OF EXTRACTS AND FRIEDELIN (MARKER)



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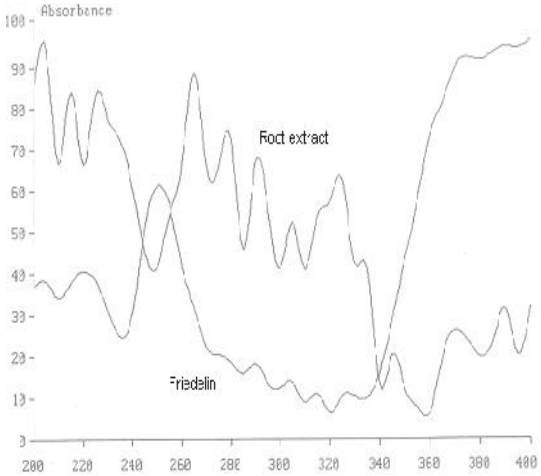
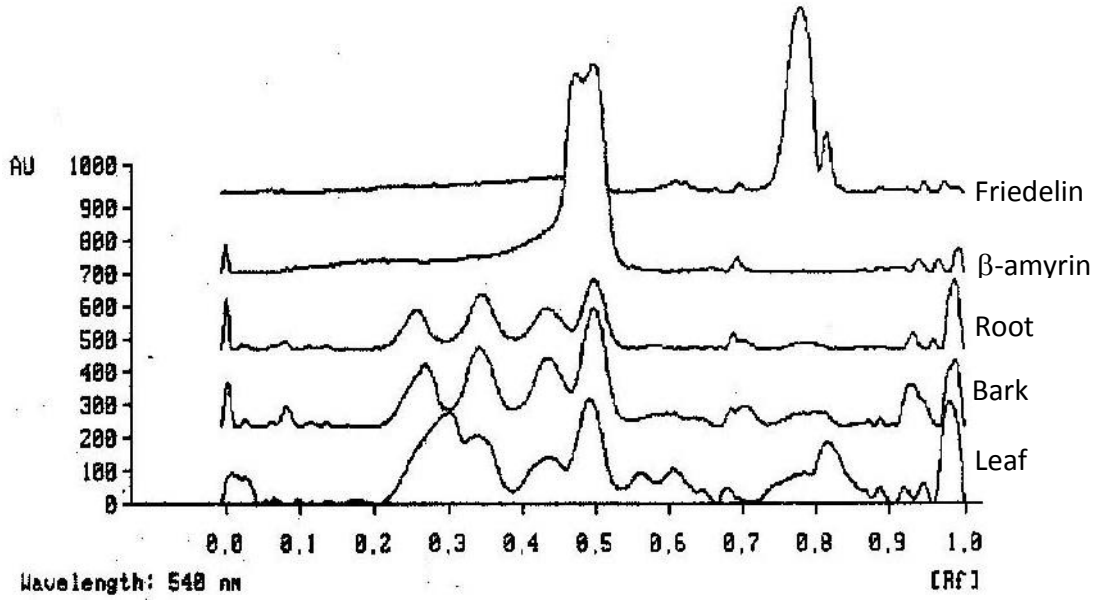


Figure 6. OVERLAIN DENSITOGRAM OF EXTRACTS AND MARKERS



DISCUSSION

The alcohol soluble extractive value was found to be higher compared to extractive values with other solvents in all parts of plant studied which proves the presence of phenolic compounds, tannin which are easily soluble in alcohol. Qualitative phytochemical test performed in the present study showed the presence of terpenoid and steroid in petroleum extract and glycosides in chloroform extract. Alcohol and aqueous extract of leaf, stembark and root showed the presence of tannin, phenols, flavonoids and saponin. Alkaloids were found to absent in all the tested extracts. Fluorescence analysis

of powder of leaf, stembark and root of the plant with different reagent as well as those of extracts found to have diagnostic value in identification of the phytoconstituents. The therapeutic power of elements was recognized in traditional system of Indian and Chinese medicine. In recent years, health care scientist and nutritionist have realized significant benefits of elements in human health.[12]The results of elemental analysis of biologically important elements were found to be considerable amount and may be directly or indirectly helpful in the management of many diseases. All the heavy metals under study, were found within the permissible limit as per WHO (World Health Organization**) [13]** and FDAC (Food and Drug Administration) i.e. for mercury (1 ppm), lead (10 ppm) and arsenic (10 ppm). The concentration of tannin, phenolic compounds and flavonoids were found to be in appreciable amount in ethanolic extract of plant. The content of vitamins such as vitamin C and Vitamin E are also found to be significant in the plant.Superimposibility of the UV spectrum of β-amyrin and friedelin with that of the corresponding spot in leaf, stembark and root shows the presence of β-amyrin in all the three extracts of the plant and the presence of friedelin in leaf extract alone. So in the present study the HPTLC profile confirms the presence of β-amyrin in all the parts of the plant and friedelin in leaf alone.The data retrieved from the observations have been formulated into a diagnostic protocol of *Dichrostachys cinerea*. The study thus contributes to our knowledge of scientific standardization of the drug of traditional claims

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