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Research Article

TLC, HPTLC and Quantitative Estimation of Acetone, Methanolic and Hydro-Alcoholic Extract of Stem Bark of *Bauhinia purpurea* Linn.

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ABSTRACT

To present study was deals with the TLC,HPTLC and Quantitative analysis of successive extract of stem bark of *Bauhinia purpuria Linn*, during the study firstly successive extraction with acetone, methanol and maceration of hydro-alcoholic extract and phytochemical study of these extract was done and secondary metabolite were observed like protein, carbohydrate, flavanoids and phenolics. the extracts were subjected to TLC and HPTLC studies to estimate number and type of phytoconstituents present in it. Number of solvent systems were tried, however good resolution was obtained in the optimized solvent system. and Quantitative estimation of acetone, methanol and hydro-alcoholic extracts were carried out, The method described by Bradford uses a different concept that is the capacity of proteins to bind a dye, quantitatively, Estimation of total carbohydrates by Phenol sulfuric acid method, total soluble phenolics in the extracts can be determined with Folin-Ciocalteau reagent and flavanoids was determined by using Quercetin. The constituents of all these extracts were quantified with spectrophotometer methods. The total proteins in acetone, methanol and hydro-alcoholic extracts were found to be 38.97, 11.92 and 11.02 mg/g, The total carbohydrates in these extracts were found to be 72.26, 319.27, and 313.60 mg/g, The total phenolics in these extracts were found to be 462, 775 and 441.11 mg/g ,and The total flavonoids in acetone, methanol and hydro-alcoholic extracts were found to be 239, 328.07and 186.53 mg/g of extracts respectively.

Keywords: *Bauhinia purpurea Linn*, TLC, HPTLC, Quantitative Estimation, Protein, Carbohydrate, Phenolics, Flavonoids

INTRODUCTION

Bauhinia purpurea Linn a medium sized deciduous tree, bark ashy to dark brown, nearly smooth, young parts brown-pubescent. Leaves 7.5-15 cm.long, rather longer than broad, cleft about half way down into 2 acute or rounded lobes, flowers large rosy purple commonly cultivated throughout India. The fast-growing Orchid Tree will ultimately reach 35 feet in height and width, the slender trunks topped with arching branches clothed in large, and two-lobe deciduous leaves. These flowers appear on the trees from September through November and are beautiful sight to see, creating a vivid splash of colorin the autumn landscape. Literature survey revealed that the bark of bauhinia purpurea is traditionally used as astringent in diarrhea.flower are laxative .Bark, Root and Flower mixed with rice water are used as a maturant for boils and abscesses[1]

MATERIALS AND METHOD

Plant Material

The stem barks of plant ***Bauhinia purpurea*** Linn. Were collected locally, from Bharatnagar opposite L.I.T Nagpur. It was authenticated by Prof. (Mrs.) Alka Chaturvedi, Department of Botany, Nagpur University, Nagpur. Its herbarium is deposited in the above department. (Voucher specimen no.9132).The stem bark of *Bauhinia purpurea* was dried in shade under normal environmental condition and subjected to size reduction. Such powdered drug was charged into Soxhlet apparatus and extraction was carried out with Acetone, Methanol and Maceration with Hydro alcoholic and phytochemical study of these extract was done and secondary metabolite were observed like protein, carbohydrate, flavanoids and phenolics [2-4]

Chromatographic Study[5]

TLC Study of Acetone, Methanolic And Hydro- alcoholic Extract

TLC and HPTLC were studied of acetone, methanol, and hydro-alcoholic extract of *B. Purpurea* and find out the probable number of compounds present in them. The details of the procedure are

the test samples were applied in the form of a band, the band was applied with the help of fine capillaries, keeping a minimum distance of 1 cm between the two adjacent bands. Bands were marked on the top of the plate for their identification; during the study Chromatographic rectangular glass chamber was used in the experiments. To avoid insufficient chamber saturation and the undesirable edge effect, a smooth sheet of filter paper was placed in chromatographic chamber in U shape and was allowed to be soaked in the developing solvent. Having been moistened, the paper was then pressed against the walls of the chamber, so that it adhered to the walls. The experiments were carried out at room temperature in diffused daylight.

**Author for Correspondence*

Table 1: The results of thin layer chromatography

Test extract	Solvent system	Number of Spots	R_f values
Acetone	Ethyl acetate:methanol:Glacial acetic acid (5:4:1)	06	0.24,0.32,0.42,0.57,0.68,0.85
Methanol	Ethyl acetate:methanol:Glacial acetic acid (5:4:1)	07	0.14,0.24,0.32,0.42,0.57,0.68,0.85
Hydro-alcoholic	Ethyl acetate:methanol:Glacial acetic acid (5:4:1)	06	0.14,,0.32,0.42,0.57,0.68,0.85

Table 2: Absorbance of standard for bovine albumin fraction IV

Sr.no	Bovine albumin fraction IV	Conc. (mcg/ml	Absorbance
1	1	6.6	0.2092
2	2	13.3	0.4233
3	3	20.0	0.569
4	4	26.6	0.7085
5	5	33.0	0.8476

Table 3: Absorbance of Acetone, methanol and hydro alcoholic for estimation of protein

Sr.no	Extract	Absorbance	Total protein content in mg/g
1	Acetone	0.1752	38.97
2	Methanolic	0.1119	11.92
3	Hydro- alcoholic	0.1098	11.02

Table 4: Absorbance of standard for dextrose solution

Sr.no	Conc. (mcg/ml	Absorbance
1	1.2	0.1897
2	2.4	0.3945
3	3.6	0.5816
4	4.8	0.7544
5	6.0	0.981

Table 5: Absorbance of Acetone, methanol and hydro alcoholic for estimation of Carbohydrate

Sr.no	Standard for gallic acid	Absorbance	Total carbohydrate content in mg/g
1	Acetone	0.1145	72.26
2	Methanolic	0.5144	319.27
3	Hydro- alcoholic	0.2138	313.

Table 6: Absorbance of standard for Gallic acid

Sr.no	Conc. (mcg/ml	Absorbance
1	50	0.053
2	100	0.087
3	150	0.134
4	250	0.247
5	500	0.497

Table 7: Absorbance of Acetone, methanol and hydro alcoholic for estimation of Phenolics

Sr.no	Standard for gallic acid	Absorbance	Total phenolics content in mg/g
1	Acetone	0.0838	462
2	Methanolic	0.1085	775
3	Hydro- alcoholic	0.0805	441.11

Developing solvent system

A number of developing solvent systems were tried, but the satisfactory resolution was obtained in the solvent systems mentioned in table. After development of plates, they were air-dried and numbers of bands were noted & RF values were calculated. Bands were visualized by spraying 10 % methanolic sulphuric acid. The Rf value was calculated as follows

The R_f value was calculated as follows

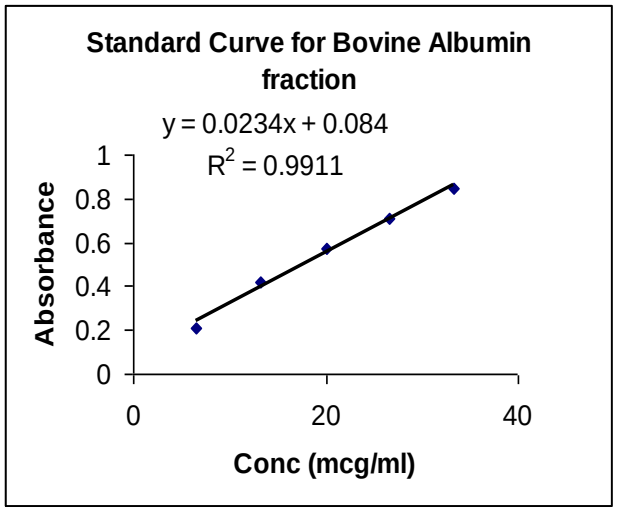


Figure 1: Standard curve for Bovine Albumin

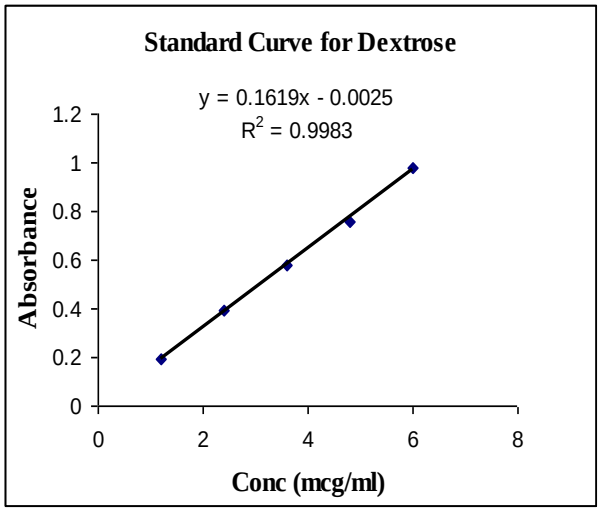


Figure 2: Standard curve for dextrose

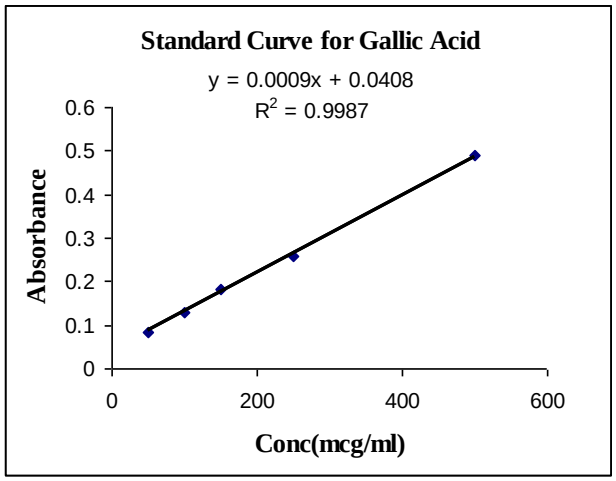


Figure 3: Standard curve for Gallic acid

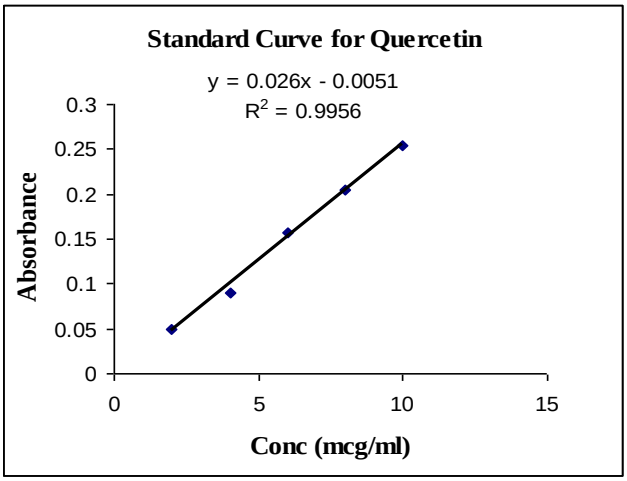


Figure 4: Standard curve for Quercetin

$$R_f = \frac{\text{Distance traveled by the sample}}{\text{Distance traveled by the solvent}}$$

HPTLC Study of Acetone, Methanolic And Hydro-alcoholic Extract

High performance thin layer chromatography study of extract *B. Purpurea*

Selection of Solvent system: The solvent system used in HPTLC for all the extracts were the same as used in TLC.

Preparation of sample: 1mg extract was dissolved in 10ml of ethanol (100µg/ml)

Application of sample: Samples were applied (5 and 10µl) in precoated plates with the help of Linomet IV applicator.

Development of Chromatogram

A rectangular twin trough glass chamber was used in the experiment. To avoid insufficient chamber saturation and the undesirable edge effect, a smooth filter paper was placed in the glass chamber and was allowed to be soaked in the developing solvent. The moistened paper was pressed against the walls of the chamber so that it adheres to the walls. The chamber was allowed to saturate for 30 minutes before use. The experiment was carried out at room temperature in diffused day light.

Procedure: The plate was dipped in a saturated chromatographic chamber containing the solvent system and was allowed to elute upto 8 cm and was air dried. The bands were scanned in CAMAG TLC scanner-3.

Chromatographic condition

Following are the chromatographic conditions required to get an effective resolutions by selected mobile phase.

Stationary phase: HPTLC precoated, silica gel G 60 F_{254} (Merck,)

Size: 5 x 10 cm

Developing chamber: Twin trough glass chamber

Mode of application: Band

Band size: 4 mm

Separation technique: Ascending

Temperature: 20 ± 5^{O}C

Saturation time: 30 min

Scanning wavelength: 254nm, 366 nm.

Scanning mode: Absorbance/Reflectance

Quantitative analysis of extracts

Estimation of total proteins[6]

The protein in the solution can be measured quantitatively by different methods. The method described by Bradford uses a different concept that is the capacity of proteins to bind a dye, quantitatively.The assay is based on the ability of proteins to Coomassie blue G 250 and form a complex whose extinction coefficient is much greater than that of

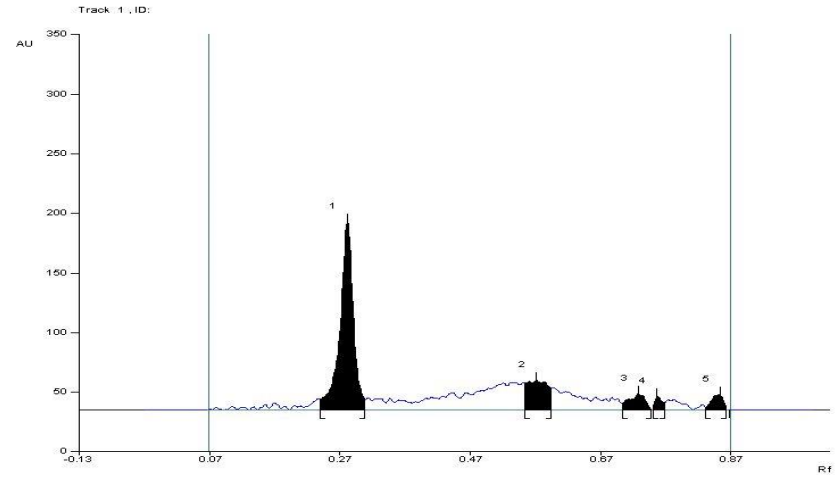
the free dye, Dye concentrate Coomassie brilliant blue G250 (100 mg) was dissolved in 50 ml of 95% ethanol. To it 100 ml of O-phosphoric acid and 50 ml of distilled water were added (stock solution). Concentrated dye solution (1volume) was mixed with four volumes of distilled water for use (working standard). and Phosphate-buffered saline (PBS) pH 7.4 was prepared, Disodium hydrogen phosphate (2.38 g), 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride were dissolved in sufficient of distilled water to produce 1000 ml and standard curve of Bovine Albumin fraction V

Table 8: Absorbance of standard for Quercetin

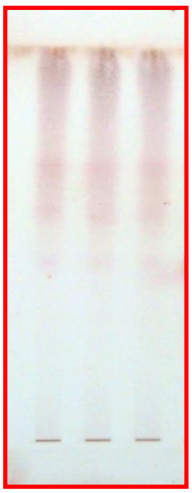
Sr.no	Conc. (mcg/ml	Absorbance
1	2	0.0502
2	4	0.0905
3	6	0.1567
4	8	0.2049
5	10	0.2534

Table 9: Absorbance of Acetone, methanol and hydro alcoholic for estimation of Flavonoids

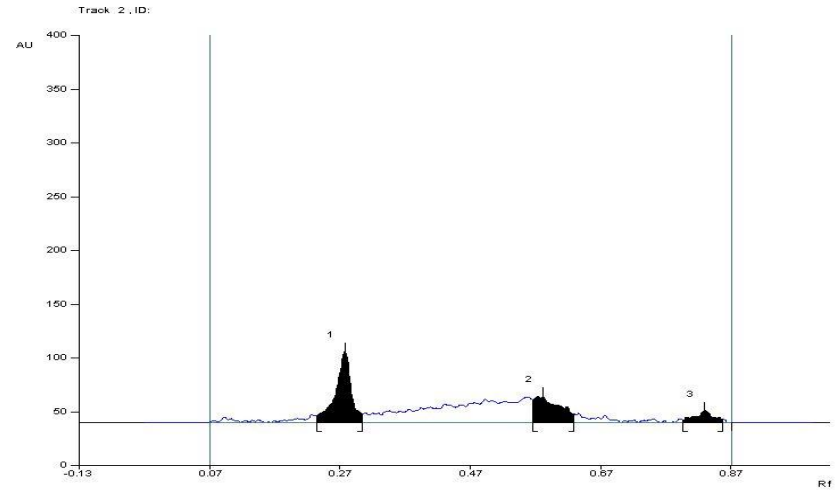
Sr.no	Standard for gallic acid	Absorbance	Total flavonoids content in mg/g
1	Acetone	0.0571	239
2	Methanolic	0.0802	328.07
3	Hydro- alcoholic	0.0434	186.53



TLC plates scanned at 254nm



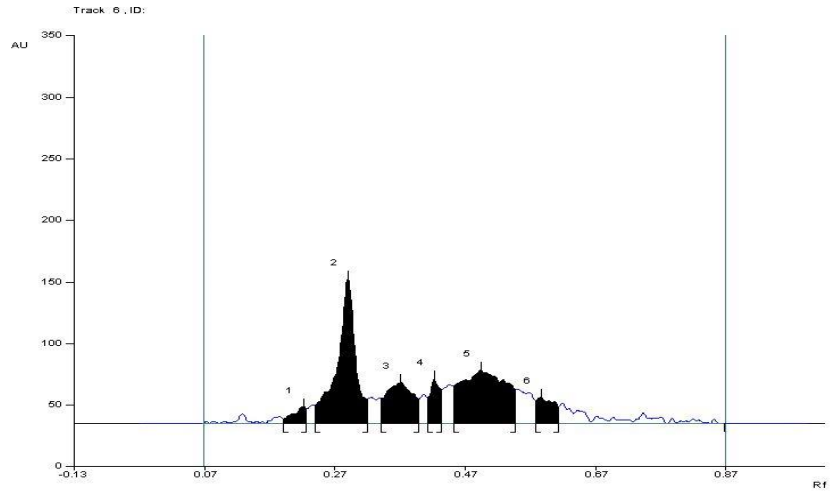
5 compounds was found having R_f value 0.28 0.57, 0.73 0.76, 0.86



TLC plates scanned at 366

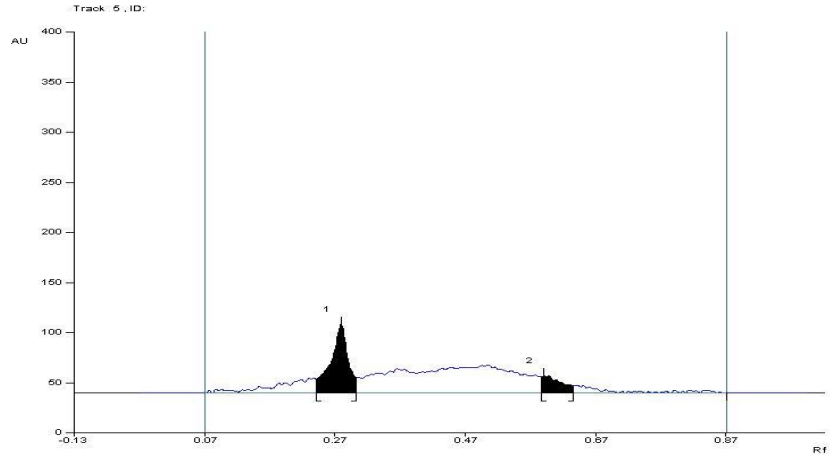
3 compound was found having R_f value 0.28, 0.59, 0.83

Figure 5: HPTLC of acetone extract of *Bauhinia purpurea* stem bark



TLC plates scanned at 254nm

6 compound were separated having R_f value 0.23, 0.29, 0.37, 0.43, 0.50, 0.59



TLC plates scanned at 366

2 compound were separated having R_f value 0.28, 0.59

Figure 6: HPTLC of methanolic extract of *Bauhinia purpurea* stem bark

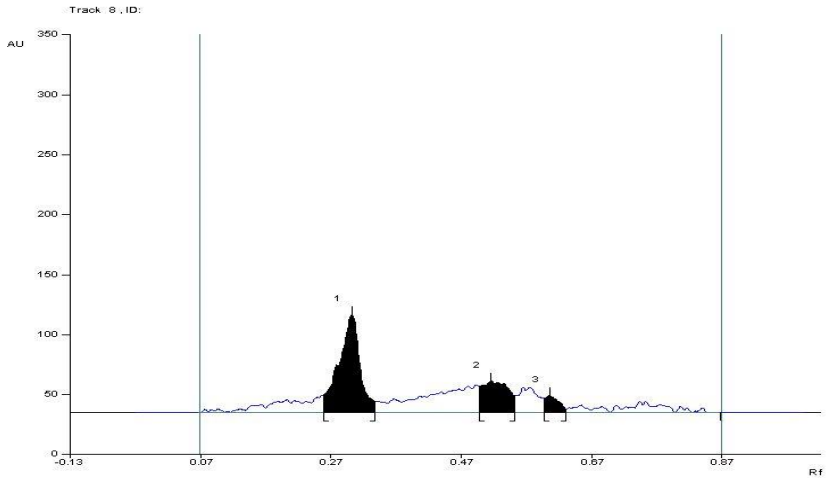
(50mg) was dissolved in 50 ml of PBS (stock solution). From this 10 ml solution was diluted to 50 ml with PBS (working standard). Further 0.2, 0.4, 0.6, 0.8 and 1.0ml of the working standard were taken and their volume was made up to 1ml with PBS, followed by the addition of 5 ml dye solution. After 5 min the absorbance was read at 595 nm. The total amount of proteins present in the sample solution was calculated using the standard graph and Preparation of sample: Extract (10 mg) was dissolved in 100 ml of PBS and to 1 ml of this solution 5 ml of dye solution was added. After 5 min the absorbance was read at 595 nm calculated by Total proteins= (conc. extrapolated from std. Curve (x)/conc. of sample)*100

Estimation of total carbohydrates by Phenol sulfuric acid method[7]

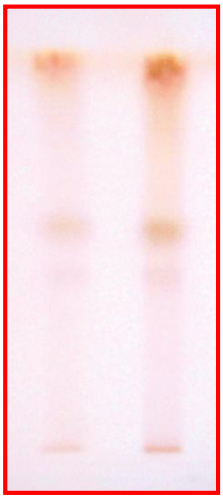
In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This forms a green colored product with phenol and absorption maximum at 490 nm.Plant material (100 mg) was hydrolyzed with 5 ml of 2.5 N HCl acid for three hours on water bath and cooled at room temperature and filtered. The solution was neutralized with sodium carbonate until the effervescence ceased and the volume was made up to 100 ml with water .The solution was centrifuged and supernatant was collected. Of the working standard 0.2, 0.4, 0.6, 0.8 and 1 ml was pipetted out in series of test tubes. Similarly of the sample solution 0.1 and 0.2 ml was pipetted out in two separate test tubes. The volume was made up to 1 ml with water and 1 ml of water was set as a blank. Phenol solution (1 ml) and 96% sulfuric acid (5 ml) was added to each test tube. After n10min the contents in the test tubes were shaken and placed in water bath at 25-30 ^{0}C for 20 min. The color was read at 490 nm.The total amount of carbohydrate present in the sample solution was calculated using the standard graph and determine by total carbohydrates= (Conc. extrapolated from std. Curve (x)/conc. of sample)*100

Determination of total phenolic conten [8]

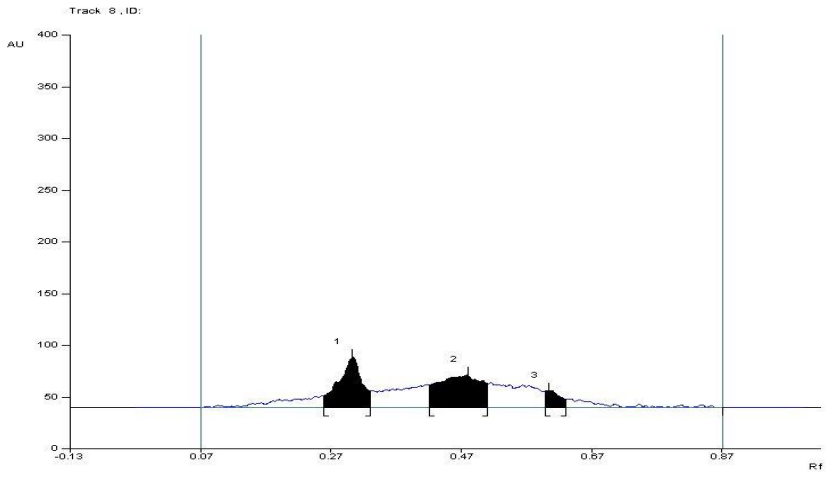
Phenols are the aromatic compounds with hydroxyl groups are widespread in plant kingdom. They occur in all parts of the plants. Phenols are said to offer resistances to



TLC plates scanned at 254nm



3 compound was found having R_f value 0.31, 0.52, 0.61



TLC plates scanned at 366

3 compound was found having R_f value 0.31, 0.48, 0.6

Figure 7: HPTLC of Hydro- alcoholic extract of *Bauhinia purpurea* stem bark

diseases and pests in plants. Grains containing high amount of polyphenols are resistant to bird attack. Phenols include an array of compounds like tannins and flavonoids etc. Total soluble phenolics in the extracts can be determined with Folin-Ciocalteau reagent according to the method of Slinkard and Singleton. Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue color complex (molybdenum blue). total soluble phenolics in the extracts were determined with Folin-Ciocalteau reagent according to the method of Slinkard and Singleton using gallic acid as a standard phenolic compound. 1.0 ml of extract solution containing 1.0 g extract in a volumetric flask was diluted with 46 ml of distilled water. 1.0 ml of Folin-Ciocalteau reagent was added and the content of the flask mixed thoroughly. 3 min later 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm. The concentration of total phenols was expressed as mg/g of dry extract .The concentration of total phenolic compounds in the extract was determined as mg of gallic acid equivalent using an equation obtained from the standard gallic acid graph Standard (gallic acid) preparation by Gallic acid (10 mg) was dissolved in 10ml of distilled water. To 1, 2.5, 5, 7.5, 12.5 and 25 ml of this solution in a volumetric flask was diluted with 46 ml of distilled water. 1.0 ml of Folin-Ciocalteau reagent was added and the content of the flask mixed thoroughly. 3 min later 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm and calculated by total phenolics = (Conc. extrapolated from std. curve(x)/conc. of sample)*100

Determination of Flavonoids [9]

Powdered plant material (1 g) was homogenized with 20 ml of extracting solvent (methanol-water-acetic acid, 140:50:10, V/V) and filtered into volumetric flasks. Volumes were adjusted to100 ml by addition of extracting solvent. Aliquots of 2.5 ml were transferred into 50 ml volumetric flasks and their volumes made up with water (analyzed solutions.) To each 10 ml of analyzed solution, 2 ml of water and 5 ml of $AICl_3$ reagent were added (133mg crystalline aluminum chloride and 400 mg

crystalline sodium acetate were dissolved in 100 ml of extracting solvent) and absorbances recorded at 430 nm against a blank (10 ml of analyzed solution plus 5 ml of water). The amount of flavonoids was calculated as quercetin equivalent from the calibration curve of quercetin standard solutions, and expressed as mg quercetin/100 g of plant material and calculated by total flavonoids = (Conc. extrapolated from std. curve(x)/conc. of sample)*100

RESULT AND DISSCUSION

TLC Study of acetone extract 6 component were separated and Rf value is 0.24,0.32,0.42,0.57,0.68,0.85 in mobile phase of Ethyl acetate:methanol:Glacial acetic acid, Methanol extract 7 component were separated and Rf value is 0.14,0.24,0.32,0.42,0.57,0.68,0.85 in mobile phase of Ethyl acetate: methanol: Glacial acetic acid, and Hydro alcoholic extract 6 component were separated and Rf value is 0.14,0.32,0.42,0.57,0.68,0.85 in mobile phase Ethyl acetate:methanol:Glacial acetic acid (Table 1).

Estimation of total proteins

Estimation of total proteins was done; the total amount of proteins present in the sample solution was calculated in acetone, methanol and hydro alcoholic extracts of *B. purpurea* were found to be 38.97, 11.92 and 11.02 mg/g respectively. (The results are expressed in terms of Bovine albumin fraction V that was used as standard, refer table no 2, 3 & fig no.1)

Estimation of total carbohydrates

Estimation of total carbohydrates by Phenol sulfuric acid method and total carbohydrates in acetone, methanol and hydro alcoholic extracts of *Bauhinia purpurea* were found to be 72.26, 319.27, and 313.60 mg/g respectively. (The results are expressed in terms of dextrose that was used as standard; refer table no. 4,5 & fig no.2)

Estimation of total phenolic content

Estimation of total phenolic content and total soluble phenolics in the extracts and the total phenolics in *Bauhinia purpurea* were found to be 462, 775 and 441.11 mg/g of powdered plant material respectively. (The results are expressed in terms of gallic acid that was used as standard; refer table no. 6,7 & fig no.3).

Estimation of total flavonoids

Estimation of total flavonoids in acetone, methanol and hydro alcoholic extracts of *Bauhinia purpurea* were found to be 239, 328.07 and 186.53 mg/g respectively. (The results are expressed in terms of quercetin, which was used as standard refer table no. 8,9 & fig no.4).

High Performance Thin Layer Chromatographically Study

HPTLC Study of acetone extract at scanned at 254nm -5 compounds was found having R_f value 0.28 0.57, 0.73 0.76, 0.86, TLC plates scanned at 366, 3 compound was found having R_f value 0.28, 0.59, 0.83(Figure 5.1, 5.2).

TLC plates scanned at 254nm 6 compound were separated having R_f value 0.23, 0.29, 0.37, 0.43, 0.50, 0.59 and TLC plates scanned at 366 2 compound were separated having R_f value 0.28, 0.59 in Methanolic extract(Figure 6.1,6.2)

Hydro alcoholic extract TLC plates scanned at 254nm 3 compound was found having R_f value 0.31, 0.52, 0.61, TLC plates scanned at 3663 compound was found having R_f value 0.31, 0.48, 0.61 (Figure 3).

ACKNOWLEDGEMENTS

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