Physicochemical and Phytochemical Analysis of Bauhinia variegata Modern analytical HPTLC Fingerprinting.

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ABSTRACT

Bauhinia variegata Linn. belonging to the family leguminosae is a medium sized, deciduous tree, found throughout India. In folk medicine, the plant is considered to be used to treat gastric and blood disorders and as liver tonic diabetic. In present study the detailed pharmacognostic study and screening for leaf and bark of is carried out to lay down the standards for its quality control purposes in future standardization studies. Its includes standardization parameters i.e organoleptic, physicochemical evaluation, preliminary phytochemical screening, HPTLC determination and total phenolic content. The data obtained can use for its quality control studies.

Keywords: *Bauhinia variegata*,poly phinol, HPTLC fingerprints, Phytochemical.

INTRODUCTION

Bauhinia variegata named orchid tree, belongs to the family leguminosae, grows 10-12 meter tall with a spreading crown of briefly deciduous leaves which are 10-20 cm across and rounded with lobed ends and heart shaped bases. “Kachnar” is an herbaceous plant, reaching up to 6-12 meters. The leaves are shaped a little like cow’s hoof and white to pinkish flowers Bauhinia variegata also known as Kachnar (Hindi), Mountain Ebony (English) is amedium sized deciduous tree distributed in sub-Himalayan tract and outer Himalaya of Punjab. The genus *Bauhinia* were used in traditional medicine for their interesting biological activities such as, anti-diabetic, anti-inflammatory, antimicrobial, analgesic, astringent and diuretic effects [1]. *Bauhinia Variegata* widely distributed plant for the Leprosy, Menorrhagia, Impurites of blood, Tuberculous gland, wound, ulcers and asthma etc. *Bauhinia Variegata* is an indigenous medicinal plant with pharmacological properties similar to rasayanas. The bark powder of the plant is a major ingredient of the herbal tonic Kanchanr guggul, an Ayurvedic medicine. Bauhinia contained many kinds of chemical constituents, primarily including flavonoids, steroids, terpenoids, tannins, lactones, glycolipids, glycosyl steroids and quinines [2, 3]. Five flavonoids isolated from the different parts of *Bauhinia* has been identified as quercetin, rutin, apigenin and apigenin 7-O-glucoside [4,5,]. It was reported the detection of insulin-like antigens in a large range of species utilizing a modified ELISA plate assay and Western blotting. [6]. In

present article pharmacognostic standards of the leves and stem bark of *Bauhinia variegata* Linn. were studied. These standards are of utmost importance not only for finding out genunity, but also in detection of adulterants in marketed drugs. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.[7]

MATERIALS AND METHODS

Plant Material:

Leves and Bark of bauhinia variegata Linn were collected from herbal garden and samples get identitified from the help of herbarium of Deendayal Research institute, Satna, M.P.

1.PHYSICOCHEMICAL EVALUATION

1. Total Ash: Accurately weighed 2g of powdered drug was taken in a tarred silica dish and ignited at temperature not exceeding 450°C until it became white (carbon free). Cooled in desiccator and weighed. Finally, percentage of total ash content was calculated with reference to air dried drug.

2. Acid Insoluble Ash:

The total ash obtained from 2g of bark and leaf sample were boiled with 25ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected on Gooch crucible, washed with hot water and ignited to obtain constant weight. The percentage of amount of acid insoluble ash was calculated with reference to air dried drug.

3. Alcohol Soluble Extractive value

2 g powdered drug was placed in a conical flask and macerated with 100 ml of Alcohol (90% v/v) for 6 hours, with frequent shaking and then allowed to stand for 18 hours. filtered through What mann filter paper. 10 ml of filtrate was transferred to flat bottom dish and solvent was evaporated on a water bath. Cooled it in desiccators for 30 minutes and finally weighed. The content of extractable matter air-dried material was calculated.

4. Water Soluble Extractive Value

2 g powdered drug was placed in a conical flask and macerated with 100 ml of water for 6 hours, with frequent shaking and then allowed to stand for 18 hours. filtered through What mann filter paper. 10 ml of filtrate was transferred to an evaporating dish and solvent was evaporated on a water bath. Cooled it in desiccators for 30 minutes and finally weighed. The content of extractable matter air-dried material was calculated.

6. Ether soluble extractive

2 g powdered drug was placed in a conical flask and macerated with 100 ml of water for 6 hours, with frequent shaking and then allowed to stand for 18 hours. filtered through Wha tmann filter paper. 10 ml of filtrate was transferred to an evaporating dish and solvent was evaporated on a water bath. Cooled it in desiccators for 30 minutes and finally weighed. The content of extractable matter air-dried material was calculated.

7. Loss on drying

Weighed 2 gm of the drug powder in a dried Petridis. The sample was heated in an oven at a temp. 105^0C and this procedure were repeated until constant weight of sample was obtaine After

drying was completed, the Petridis was allowed to cool in desiccators. The loss on weight in percentage of air-dried material was calculated.

2.PHYTOCHEMICAL EVALUATION:

Phytochemical screening:

Phytochemical analysis of aqueous and alcoholic extracts, of the drug was carried out by employing standard protocols [8-9] for determining the presence and/or absence of Phytochemicals : following tests are performed.

1. Alkaloids:
   (Dragendorff's test): Dissolve a few ml of alcoholic or aqueous extracts of drug in 5 ml of distilled water, added 2 ml HCL until on acid reaction, occurs then added 1ml of dragendorff's reagent, an orange or orange red ppt is produced immediately.
   (Wagner test): acidify 1 ml of alcoholic extract of the drug 1.5% v/v of HCl and few drop s of wagner reagents, a yellow or brown ppt is formed.
2. Flavanoids:
   (Shinoda test): In test tube containing 0.5 ml of alcoholic extract of drug, added 5-10 drops of dil HCl followed by small pieces of mg in the presence of flavanoid pink, reddish pink or brown color is produced
3. Tri- terpenoids:
   (Libarmann Burchard test): Added 2ml of acetic anhydride solution to 1ml of petroleum ether extract of drug in chloroform followed by 1 ml of conc. H_2SO_4, a violet colored ring is formed indicate the presence of triterpenoids.
4. Carbohydrate:
   (Anthrone test): To 2ml of anthrone test solution added 0.5 ml of aqueous extract of drug , a green or blue color indicates the presence of carbohydrate.
   (Fehling test): To 2 ml of aqueous extract of drug, add 1 ml of mix of equal part of Fehling solution a and B and boil the content of the test tube for few minute are or brick red ppt. is formed.
5. Protein:
   (Biurate test): To 1 ml of hot aqueous extract of drug, added 5-8 drops of 10% w/v NaoH solution followed by 1-2 drops of 3% w/v $CuSO_4$ solution, a red or violet color is obtained
   (millon test):dissolve small quantity of aqu.exrect of drugs 1ml of distilled water and 5-6 drops of millions reagent .a white ppt is formed which turns red on heating.
6. Resin:
   Dissolved the aqueous extract in 1 ml of acetone and poured the solution into 5 ml of distilled water, turbidity indicates the presence of resin.
7. Saponins:
   (Foam tests): in test tube containing about 5 ml of an aqueous extract of drug, add drops of sodium bicarbonate, shacked it vigorously and left for few minutes, honey comb like structure is formed.
8. Tannins:
   To 1-2 ml of extracts of the drug, added few drops of 5% $FeCl_3$ solution, a greenish color indicate the presence of gallotannin, and white brown color for tannin.

9. Steroids: (Salkowski tests): Added 1 ml of conc. H_2SO_4 to 2 ml of chloroform extracts of the drug carefully, forum the side of test tube a red color is produced in the chloroform layer.

3.HIGH PERFORMANCE THINLAYER CROMOTOGRAFY [HPTLC] STANDRIZATION.

HPTLC of Methanolic extract of leaves and stem bark were carried out by using Tolune: ethyl acetate (8:1) and as solvent system. 4 ul of the test solutions sample drugs were spotted on pre coated silica gel aluminium plate 60F-254 (5cm×10cm) using Camag Linomat V sample applicator and 100ul Hamilton syringe. Silica gel Plats were developed in Camag development chamber followed by photodocumentation in Camag photoreprostar with CATS software.[10-11] And Developed fingerprint profile would serve as a reference standard for quality evaluation and standardization of the formulation with same drug. Rf values and colour of the bands from separated compounds.

4.TOTAL PHINOLIC COMPOUND

Accurately 2 gm powdered drug was taken and extracted with 75ml of 50% methanol by cold maceration for 2 hrs with intermittent shaking. Solution filtered and volume was made up to 100 ml**.** From the above extract, 0.1ml was pipette out into a 25 ml volumetric flask and 10 ml of distilled water was added followed by 1.5 ml of folin ciocalteu reagent. After 5mins 1ml of 20% sodium carbonate solution was added and volume was made up to 25 ml with distilled water and incubated for 30mins. After 30 mins absorbance was recorded at 765nm[12].
10 mg of standard Gallic acid was accurately weighed and dissolved in 100 mL distilled water in a volumetric flask (100µg/mL of stock solution) and then pipette out 0.5 to 2.5mL of aliquots into 25mL volumetric flasks from the above prepared stock solution. Then 10 mL of distilled water and 1.5mL of Folin Ciocalteu reagent. After 5 min, 1mL of 20% sodium carbonate was added, and then distilled water was added to make the volume up to 25 mL. After 30 min, absorbance at 765nm was recorded and calibration curve of absorbance v/s concentration was recorded.Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass). The total phenolic contents of the methanolic extract were calculated by using standard calibration curve.

RESULTS AND DISCUSSION

In present study the stem bark of *Bauhinia variegata* Linn was evaluated for its physicochemical and phytochemical aspects along with HPTLC determination which revealed the following results. Organoleptic parameters revealed that the powder of leves and bark of *Bauhinia variegata* are greenish,reddish and brownish in color, with the characteristic odour, bitter taste and fine and Hard, longitudinally striated texture(table-1). Quality tests for both parts of drug powder were performed for moisture content, ash content, acid insoluble ash, water soluble extractive, alcohol soluble extractive, and petrolium ether extractive were found to be within standard ranges. The Results of physicochemical analysis are given in (Table-2). The total ash value is anindicative of total amount of inorganic material after complete incineration and the

acid insoluble ash value is an indicative of silicate impurities, which might have arisen due to improper washing of drug. Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards [14]. The extractive values names water soluble and alcohol soluble indicates the amount of active constituents in given amount of plant material when extracted with respective solvent. The loss on drying value obtained is an indicative of amount of moisture content present in the drug. pH of 10%w/v solution revealed that the formulations are acidic. The less value of moisture content could prevent bacterial, fungal or yeast growth [15]. The results of physicochemical parameters of ingredients no 01 and 02 are table in (Table-1&2) separately and compliance with Ayurvedic pharmacopoeia of India [16]. The results of preliminary phytochemical screening showed the presence of carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins, sterols and tannins, in the methanolic extract of the drugs (table-3) which could make the drug useful for treating different ailment as having a potential of providing useful drugs for human use.

HPLTC is an important analytical tool in the separation, detection and estimation of different classes of natural products. HPTLC fingerprint profile of the powders are depicted in figure (Plate 1,2,3,4 Fig -1,-2) indicates the presence of various spots with different colour at different Rf values. Developed fingerprint profile would serve as a reference standard for quality evaluation and standardization of the formulation with same drug. Rf values and colour of the bands from separated compounds are recorded in (Table – 4,5)

The total phenolic contents of methanolic extract were measured by UV spectrophotometric method. The total content of phenolic compounds was found to be 0.9923 mg/gm in methanolic extract of *Bauhinia variegata* Linn. The presented values are Mean ±S.D. of triplicate determinations. The total phenolic content was expressed as gaillic acid equivalent in mg/gm of the extract[16-17].The standard calibration curve was used to calculated the total phenolic contents in the methanolic extract of the drugs. (fig-1) Phenolic compounds are most widely occurring groups of phytochemicals and derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants. These compounds are secondary metabolites which have vital role in reproduction and growth, gives protection against harmful predators and pathogens. Therefore, quantitative analysis of these compounds is very important to check the quality of drug.

CONCLUSION

Bauhinia variegata Linn is extensively used in the traditional system of medicine for treatment of number of diseases and considered as an important medicinal plant. As per literature survey very less work has been reported on this variety. From the present investigation various standardization parameters such as organoleptic, physiochemical parameters like total ash, acid insoluble ash, water & alcohol soluble extractive values, loss on drying, Phytochemical analysis and HPTLC fingerprinting modern analytically profiling were carried out as perWHO/Ayurvedic Pharmacopoeial standards. Presence of various phytoconstituents can serve as basis for screening of different pharmacological activities, investigation and further research. The results of present study showed satisfactory results, but the efficacy of the products can only be judged by doing the pharmacology.

TABLE 1: ORGANOLEPTIC CHARACTERS

SN	PARAMETER	OBSERVATION	
		leaves	bark
1	color	Greenish	Brownish,reddish,pinkish
2	Odour	Characteristic	Characteristic
3.	taste	biter	bitter
5	Texture	Fine,soft	Hard, longitudinally striated

TABLE 2: PHYSICOCHEMICAL EVALUTION

SN	PARAMETER	STANDRD VALUE	
		leaves	bark
1	Total Ash	9.88	17.05
2	Acid-insoluble Ash	3.44	7.94
3	Alcohol soluble Extractive	13.65	17.99
4	Water soluble Extractive	13.28	27.76
5	Petroium ether extractive	2.80	4.65
6	Loss on Drying	4.48	5.8

TABLE 3: PHYTOCHEMICAL SCREENING

PHYTOCHEMICAL COMPOUND	TEST OR OBSERVATION	RUSULTS	
Carbohydrates	Anthrone test, Fehling test	+ +	+ -
Resin	Acetone	-	+
Saponins - -	Foam tests	+	-
Terpenoids +	Libarmann, Burchard test	- -	- -
Alkaloids	Dragendorff's test, wagners test	+ +	+ +
Steroids	Salkowski tests,reaction	- -	- +
Flavanoids	Shinoda test	+	+
Tannins	5% $FeCl_3$	+	-
Proteins	Biurate test,millon test	+ +	+ +

TABLE -4 HPTLC FINGER PRINTING RF VALUES AND COLOUR BAND OF LEAVE
SOLVENT SYSTEM- *n-Hexane: Ethyl acetate (8:2)*

Sn.	254nm	366nm	After darivitazed	Day light
1	0.76	0.04	0.10	0.10
2	0.79	0.27	0.64	0.65
3	0.82	0.53	0.81	0.81
4	0.89	0.84	0.95	0.95

Sn.	254nm	366nm	After darivitazed	Day light
1	Black	Pink	Grew	Grew
2	Black	Red	Violet	Violet
3	Black	Green	Chocklety	Brown
4	Black	Red	Orange	Grew

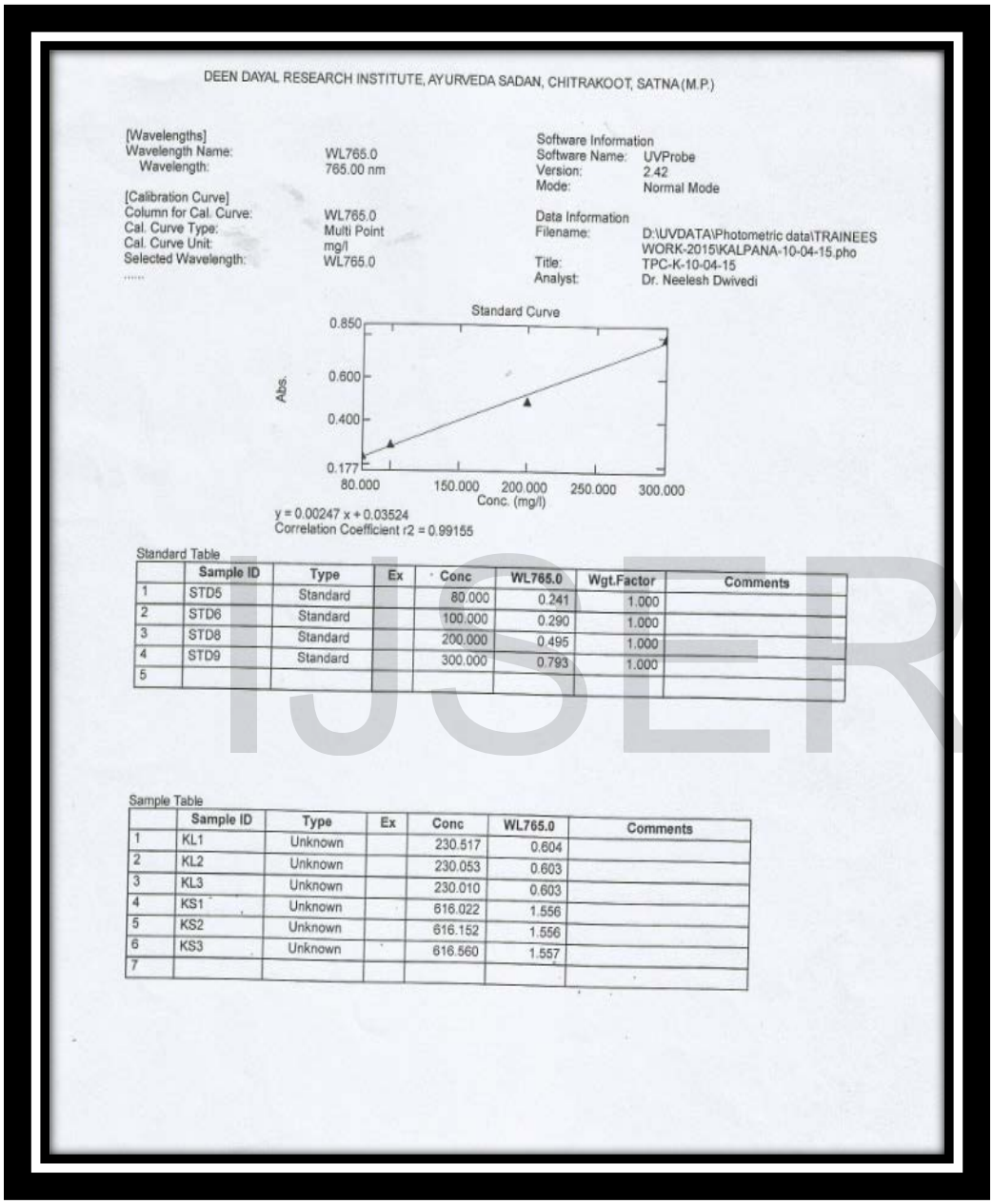
TABLE -5 HPTLC FINGER PRINTING RF VALUES AND COLOUR BAND OF STEM
SOLVENT SYSTEM- *n-Hexane: Ethyl acetate (8:2)*

Sn	254nm	366nm	After darivitazed	Day light
1	0.76	0.51	0.36	0.54
2	0.79	0.69	0.42	0.69
3	0.82	0.83	0.56	0.92
4	0.89	0.90	0.65	0.97
5		0.95	0.74	
			0.95	

Sn	254nm	366nm	After darivitazed	Day light
1	Black	Blue	Blue	Black
2	Black	Sky blue	Brown	Black
3	Black	Red	Light green	Black
4	Black	Orange	Pink	Black
5		Dark red	Blue	
			Green	

TABLE-6 STANDERD CALIBRATION CURVE FOR DETERMINATION OF TOTAL PINOLIC COMPOUND.

Fig. 1



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[Wavelengths]
Wavelength Name: WL765.0
Wavelength: 765.00 nm

[Calibration Curve]
Column for Cal. Curve: WL765.0
Cal. Curve Type: Multi Point
Cal. Curve Unit: mg/l
Selected Wavelength: WL765.0

Software Information
Software Name: UVProbe
Version: 2.42
Mode: Normal Mode

Data Information
Filename: D:\UVDATA\Photometric data\TRAINEES WORK-2015\KALPANA-10-04-15.pho
Title: TPC-K-10-04-15
Analyst: Dr. Neelesh Dwivedi

y = 0.00247 x + 0.03524
Correlation Coefficient r2 = 0.99155

Standard Table

	Sample ID	Type	Ex	Conc	WL765.0	Wgt.Factor	Comments
1	STD5	Standard		80.000	0.241	1.000	
2	STD6	Standard		100.000	0.290	1.000	
3	STD8	Standard		200.000	0.495	1.000	
4	STD9	Standard		300.000	0.793	1.000	
5							

Sample Table

	Sample ID	Type	Ex	Conc	WL765.0	Comments
1	KL1	Unknown		230.517	0.604	
2	KL2	Unknown		230.053	0.603	
3	KL3	Unknown		230.010	0.603	
4	KS1	Unknown		616.022	1.556	
5	KS2	Unknown		616.152	1.556	
6	KS3	Unknown		616.560	1.557	
7						

Fig-2 HPTLC Fingerprints leave photometric of silica plate 366 nm and 254 nm.

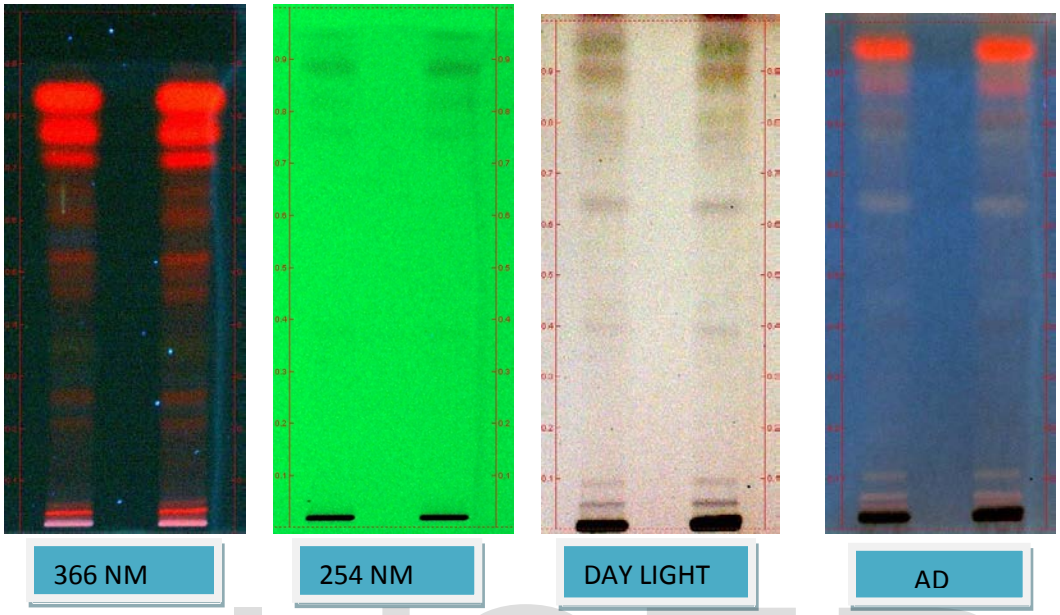
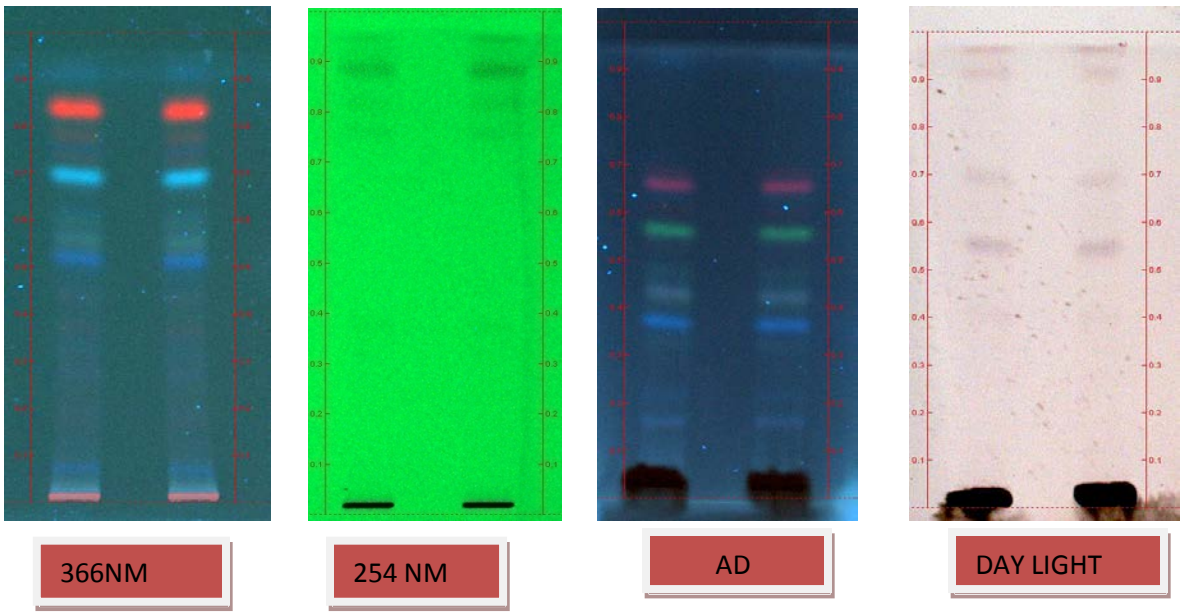


Fig-3 HPTLC Fingerprints stem photometric of silica plate 366 nm and 254 nm.



REFRENCE

1. Chu, W. L. (2005). SemBioSys Reports Positive Preclinical Results of Plant Produced Insulin. In the Plant Biotechnology Journal.
2. C.R. Azevedo, F.M.Maciel, L.B. Silva, A.T. Ferreira, M. da Cunha, O.L. Machado, K.V. Fernandes, A.E. Oliveira, J. Xavier-Filho, *Braz. J. Med. Bio. Res.*, **2006,** 39, 1435-1444.
3. M.A. Mohamed, M.R. Mammoud, H. Hayen, *Zeit. Natur. C.*, **2009,** 64, 798-808.
4. Silva, K. L. and Cechine-Filho, V. (2002). Plantas do genero Bauhinia: Composicao quimica epotencial farmacologico. Quim. Nova. 25: 121-125.
5. Rajani, G. P.,and Purnima, A. (2009). *In vitro* antioxidant and antihyperlipidemic activity of *Bauhinia variegata* Linn. Indian J. Pharmacol.,;41(5)227-32.
6. Barbosa-Filho, JM.; Vasconcelos, THC.; Alencar, AA.; Batista, LM.; Oliveira, RAG.; Guedes, DN.; Falcao, HS.; Moura, MD.; Diniz, MFFM. and Modesto-Filho, J. (2005). plants and their active constituents from south, Central, and North America with hypoglycemic activity. Rev. Bras. Farmacogn, 15: 392-413.
7. (Quality control methods for medicinal plant material by WHO; 1998)**.**
8. Anonymous. Quality control methods for medicial plant materials. WHO, Geneva. 1998.
9. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan, NewDelhi, India. 1994: 54.
10. Evans, W. C. (1989). Pharmacognosy 13th (Eds) Balliere Tindal, London, 419-420.
11. Harborne, J. B. (1984). Phytochemical methods. A guide to Modern Technique of Plant Analysis, Chapman Hall, London.
12. Anonymous, The Ayurvedic Pharmacopoeia of india,Part-II,Vol-1,first edi,(Formulation),Govt. of India,ministry of Health and Family welfare,AYUSH,New Delhi.2007.
13. McDonald S, Prenzler PD, Autolovich M and Robards K(2001). Phenolic content and antioxidant activity of olive extracts. *Food Chem.,* **73**: 73-84.
14. Chopara, S.L., Kanwar, J.S., In Analytical Agricultural Chemistry, iv edition, Kalyani publications, New Delhi, pg. 297, **1991**.
15. African Pharmacopoea, General methods for analysis. 1st edition, vol-2 (OAU/STRC) Lagos, pg-123, **1986.**
16. Anonymous., The Ayurvedic Pharmacopoeia of India., Part -I, Vol-VI, Government of India, Ministry of Health and Family Welfare, Department of ISM and H, New Delhi. **2000**.
17. Gupta Daksha, Chandrashekar1, Richard Lobo, Yogendra and Gupta Nilesh, et al. Antidiabetic activity of stem bark of *Bauhinia purpurea* Linn, Scholars Research Library Der Pharmacia Lettre., 2012, 4 (2):614-61.
18. .Kadam Prasad V., Yadav Kavita N., Navasare Vidya S., Bhilwade Sumeet K., Patil Manohar J, etal. A Phytopharmacognostic Study, International Journal of PharmaceuticalPhytopharmacol.Research., 2012, 1(6): 350-353.
19. Shekib LA Iraq EI, Abo Baker SM, et al."Studies on amylase inhibitors in some Egy legume seeds" Plant Foods Human Nutr.1988,38, 325–332.
20. Seth S.D., Sharma B, et al. Medicinal plants of India. Indian Journal of Medical Research., 2004,120: 9–11.