

"A COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDY OF ASHOKA (*Saraca asoca* (Roxb.) de Wild) TWAKA SAMPLE FROM DIFFERENT DESHAS"

Dr Hosmath Veerendra¹ , Dr. Uday Pratap Singh² Dr Hosmath Shashikala³
¹Professor in Dept. of Dravyaguna ³Rasashastra & Bhaishajya KalpanaShri J G Co-Operative Society's Hospital and Ayurvedic Medical College, Ghataprabha. ²Lecturer in Dravyaguna Shanti Ayurvedic Medical College and Hosptial Ballia UP

DOI: <https://doi.org/10.47071/pijar.2021.v06i04.01>

ABSTRACT

A Pharmacognostic and phytochemical study of **Ashoka (*Saraca asoca* (Roxb.) de Wild) twaka** collected from three different desha (Anupa, jangal and sadharana) was carried out. The Hydro-alcoholic extract were prepared and the individual extracts were subjected to the qualitative and the quantitative phytochemical analysis.

Quantitative phytochemical analysis was carried out for the major group of bioactive phyto-compounds, i.e. Total phenols, total flavonoids, total tannins and total . Qualitative phytochemical analysis of gallic acid was also carried by using High Performance Thin Layer Chromatographic technique (HPTLC) for all the samples.

Out of three sample i.e. Anup desha, Sadharana Desha and Jangal desha, However, Anup desha samples showed better bioactivity in majority of the parameters like, preliminary phytochemicals and in Total Flavonoids, Total phenolst glycosides ,total Total Tannin content. Major bioactive like gallic acid were also higher concentrations in Anup desga sample extract when compared to other deshas. Hence Anup desha is the ideal season for the collection of the Ashoka bark.

Key words: Ashoka, Anup desh, HPTLC.

Introduction :

Ayurveda, one of the most ancient sciences of the world has pioneered many concepts which are relevant even at

21st century, thus accentuating the eternity of the science. The entire science of Ayurveda is based on its three pillars-Hetu, Linga and Aushada. Among the Aushada-jnana includes

study of the Aushada Dravya in its various aspects.

Plants are one of the main sources of medicine since immemorial time. Ayurveda emphasize on use of plants as medicine. As Charakaacharya ¹ says that each and every dravya in this universe they are some medicinal property. It shows that our Acharya's are how much conscious about plants with all their aspects.

-प्रयोजनं चास्य स्वस्थस्य स्वस्थरक्षणं
आतुरस्य विकारप्रशमनं च।।-च.
सू.३०/२६

Dravyaguna Shastra is an important branch of chikitsa shastra. Ayurveda advocate two principles to maintain normal health. To maintain the health of a healthy person and to cure disease of a diseased person. Knowledge of Ahara and Aushada Dravya are important. Because of these dravya has got so much importance in the field of Dravyaguna and Chikitsa shastra.

To administer proper dravya to relieve such diseases perfect knowledge of dravya

is essential. So the knowledge of Dravyaguna Shastra is very important.

-निगण्टुना विना वैद्यो विद्वान् व्याकरणं विना
अभ्यासेन् च धानुष्कस्त्रयो हास्यस्य भाजनम्।।-रा.
नि. प्रस्तवना.९.

In Raja Nighantu, it is stated that if a

physician does not know about Nighantu a scholar without the knowledge of grammar and a person trying to use bow and arrow without proper practice all becomes a laughing stock. Here it shows the importance of the knowledge of Dravya.

As per classical literatures like Charaka Samhita¹, Astanga Hrudaya ² Susruta Samhita ³ and Raja Nighantu, which specify that the drug should be collected from appropriate desha as they contain maximum Guna's in that particular desha. This indicates the authentic knowledge about the drug potency (concentration of active principles) in various part of the earth.

The drugs are capable of producing maximum therapeutic effects when their potency is augmented by Desha, Kala, Prayojya anga etc. Desha's plays a very important role in getting the desired therapeutic efficacy such as RASA, VEERYA, VIPAKA and GUNA.

Therefore it is important to follow good collection methods to get the optimum results from crude drug. Classically different Desha's are mentioned for collection of different DRUG to get good therapeutic results.

As each and every plant possesses

**A COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDY OF ASHOKA
(*Saraca asoca* (Roxb.) de Wild) TWAKA SAMPLE FROM DIFFERENT DESHAS**

individual inherent qualities the ancient authors have advocated particular Desha for the collection of particular medicinal plants. Ancient system has always mentioned the use of fresh plants, for therapeutic purposes.

In case fresh medicinal plants are not available it can be substituted with dry form of same plant. The medicinal plant thus used has to be collected from specific desha and should preserve in proper manner. This method helps in preserving properties that are attributed to the drug.

In market one formulation is prepared by many pharmacies, so requirement of raw material is increasing day by day. As Ayurvedic formulations are marketed globally care should be taken to follow GACP⁴ (Good Agricultural and Collection Practices). Keeping this in mind, the present study was planned and carried out to compare the variations in samples collected from different desh.

MATERIALS AND METHODS

The sangrahadesh (Place of collection) of different parts of the plant are required to be analyzed under the light of modern scientific methodology. To evaluate Ashoka bark collected from

different deshas, this study was carried out in four steps. The materials utilized and the methodologies adopted are presented here under.

I. PROCUREMENT OF PLANT FROM NATURAL SOURCE :

II. ORGANOLEPTIC STUDY OF SAMPLES COLLECTED FROM THREE DESHAS:

III. PHYSICO - CHEMICAL EVALUATION COLLECTED SAMPLES:

IV. PHYTO - CHEMICAL STUDY FOR QUANTIFICATION.

I. PROCUREMENT OF PLANT SOURCE:

Ashoka (*Saraca asoca* (Roxb.) de Wilde) bark Plant identification: was done by Taxonomist based on -

- 1) Synonyms given in classics of Ayurveda^{6,7,8}
- 2) Morphology and family characters of plant.^{9,10}

Authentification:

Plant material was identified on the basis of Bark morphology at Agarkar Research Institute, Pune by, Smt. A.S. Upadhye. Three Sample of Ashoka (*Saraca asoca* (Roxb.) de Wilde) bark was collected from Three Different Deshas (Place).

Sample 1- Kolkata (Anup)

Sample 2- Ghatprabha (Sadharan)

**A COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDY OF ASHOKA
(*Saraca asoca* (Roxb.) de Wild) TWAKA SAMPLE FROM DIFFERENT DESHAS**

Sample 3- Hariyana (Jangal)

Amount of samples:

Around 2 Kg of the drug was collected in each season, washed thoroughly with water to remove physical impurities like mud and dried under shade till it gives constant weight. 50 Gm of the drug was kept apart for organoleptic study. Then the drug was made into coarse powder and kept preserved in air tight container for phyto-chemical and physic-chemical analysis.

LOCATION OF THE STUDY:

• Dr. Prabhakar Kore, Basic Science Research Center, Belgavi

II. ORGANOLEPTIC STUDY OF THREE TEST DRUGS:

Ashoka(*Saraca asoca* ⁵(Roxb.) de Wilde) bark: Macroscopic character of the collected bark was observed and compared with that of given description in classical and standard texts.

MICROSCOPIC EXAMINATION:
Ashoka(*Saraca asoca* (Roxb.) de Wilde) bark:

Requirements: Razor blade, forceps, needles, brush, watch glass, glass slides, cover slips, blotting paper, phloroglucinol, HCl, water, dropper, compound microscope.

Procedure:

Fresh bark of ASHOKA was taken for

Microscopic examination. The bark and Razor blade was made wet with water. The razor blade was taken in right hand held at right angle. Sectioning was done by moving the razor blade over the bark. The sections present on the razor blade were now transferred with a brush into the water in a watch glass.

Staining and mounting:

A thin uniform and entire section was taken and transferred with a brush to the middle of clean glass slide and a drop of Phloroglucinol was added. Section was allowed to stain for few minutes and then the excess of stain was washed off using water. Then section was mounted by placing 1-2 drops of HCL by dropper and covered with a clean thin cover glass.

POWDER MICROSCOPY:

Materials required:

Fine powder of the drugs, chloral hydrate solution, 1% safranin stain, water, watch glass, glass slides, cover slips, blotting paper, dropper, compound microscope.

Procedure:

- The fine powder of the stem bark of Ashoka(*Saraca asoca* (Roxb.) de Wilde) bark were soaked in water and 2-3 drops of chloral hydrate for few

A COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDY OF ASHOKA (*Saraca asoca* (Roxb.) de Wild) TWAKA SAMPLE FROM DIFFERENT DESHAS

minutes.

- The soaked drugs were taken to the slide with a spatula and spread neatly.
- A drop of 1% safranin stain (1g safranin in 50% alcohol) was put and left for few minutes.
- Then the cover slip was placed. Excess water was removed with the help of blotting paper and the slide was observed under the microscope.

III. PHYSICO - CHEMICAL EVALUATION

1. Determination of foreign matter:

Drugs should be free from moulds, insects, animal faecal matter and other contaminations such as earth, stones and extraneous materials.

Material required: Drug, Digital balance

Method:

Weigh 100-500gm of the drug sample to be examined or the minimum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6x).

2. Determination of Moisture by air oven:

Materials: Aluminium/stainless steel dishes with cover, air oven.

Calculation: % of Moisture $\frac{W_2 - W_3}{W_2 - W_1} \times 100$

DETERMINATION OF ASH VALUE:

Aim & Significance:

In determination of total ash values the carbon must be removed at temperature (450°C) as possible because Alkali chlorides, which may be volatile at high temperatures, would otherwise be lost. The total ash usually consists mainly of Carbonates, Phosphates, Silicates and Silica. If the total ash be treated with dil.HCl, the % of acid insoluble ash may be determined.

ESTIMATION OF TOTAL ASH CONTENT:

Total ash refers to the non-organic residue present in samples after burning and subjecting to heating about 500^o- 550^oc.

Requirements:

Silica crucible, muffle furnace, desiccator.

Calculation: Total ash % =

$$\frac{W_3 - W_1}{\text{Weight of Sample}} \times 100$$

ESTIMATION OF ACID INSOLUBLE ASH CONTENT:

Ash left over after dissolving the total ash in dilute HCl refers to acid insoluble ash and represents siliceous matter.

Reagents: 10% Hcl solution.

Calculation:

Acid insoluble ash %

**A COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDY OF ASHOKA
(*Saraca asoca* (Roxb.) de Wild) TWAKA SAMPLE FROM DIFFERENT DESHAS**

$$= \frac{W_4 - W_1}{\text{Weight of Sample}} \times 100$$

EXTRACTIVE VALUE:

Aim: The determination of Hydro-alcoholic soluble extractive is used as a means of evaluating drugs, the constituents of which are estimated by continues extraction process.

DETERMINATION OF HYDRO-ALCOHOLIC SOLUBLE EXTRACTIVE VALUE.

Materials required: Soxhlet apparatus, Rotary evaporator, Lypholyzer.

Procedure:

1. Dry powder of *Saraca asoca* is placed inside a thimble made from thick filter paper (watman's filter paper No 1).
2. The thimble is placed in Soxhlet distillation unit, which contains an extraction chamber, which is suspended above a flask containing

the solvent - hydro-alcohol and below a condenser.

3. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it is automatically emptied by a siphon side arm, with the solvent running back down to the boiling distillation flask.

PHYTOCHEMICAL TESTS:

FLAVONOIDS:

The extracts were tested for the presence of flavonoids by Shinoda's reaction (Shinoda, 1928). Ethereal solutions of the extracts were evaporated and residue dissolved in ethanol. On addition of Magnesium powder and concentrated hydrochloric acid, the development of Yellow/ red colour indicated the presence of flavonoids.

Table No1. Showing Phytochemical constituents of *Saraca asoca* sample:

Test	Method	Observation
Tannins	Ferric chloride test Dilute extract + 2ml ferric chloride solution	Dark blue colour
Alkaloids	Dragendorff's test Extract + Dragendorff's reagent	Yellow/ orange precipitate
Steroids	Leiberman Burchard test Extract + CHCl ₃ + Ac ₂ O + 2 drops of Conc. H ₂ SO ₄	Green colour
Glycosides	Molisch's Test Extract + α-naphthol + 1ml Conc. H ₂ SO ₄	Violet ring at the junction of two layers

**A COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDY OF ASHOKA
(*Saraca asoca* (Roxb.) de Wild) TWAKA SAMPLE FROM DIFFERENT DESHAS**

Reducing sugars	Fehling solution test Extract + fehling solution A & B- boiling on water bath	Brick red precipitate
Amino acids	Ninhydrine test Extract hydrolysis with HCl + 0.1% Ninhydrine solution	Violet purple colour
Starch	Add 1-2 drops of weak iodine solution to small quantity of dried powder and observe under the microscope	Blue colour starch grains
Flavonoids	Shinoda test Extract + Mg Turnings + 2 drops of Conc. HCl	Pink colour

DETERMINATION OF pH VALUE:

Materials required :

pH meter, Buffer solution, powdered drug, Distilled water, Filter paper.

The measurement of pH is generally done with a suitable Potentiometer known as pH meter (fitted with 2 electrodes, one constructed of glass and sensitive to hydrogenation activity and the other a calomed reference electrode) of 25 + 2°C.

Estimation Of Fat Content In Samples:

Fat content in samples in terms of free lipids or petroleum ether extractable lipids is estimated by using Soxhlet extraction with continuous refluxing for 14-16 hours.

Reagents: Petroleum ether 40-60°C fraction.

Apparatus: Soxhlet extraction set, isomantle thimbles.

$$\text{Calculation: \% of fat} = \frac{W_4 - W_3}{W_2 - W_1} \times 100$$

$$= \frac{\text{Weight of fat}}{\text{Weight of Sample}} \times 100$$

QUANTIFICATION OF PHYTOCHEMICALS¹¹

ESTIMATION OF TOTAL PHENOLS:

Sample Preparation and Tannins Extraction

The sample was dried at 55±10 C and ground to pass through a sieve of 1mm diameter. Tannins extraction was done using 400 mg ground sample in conical flask with 40 ml diethyl ether containing 1 per cent acetic acid (v/v) and mixed to remove the pigment material. Carefully discarded the supernatant after 5 minute and 20 ml

of 70 per cent aqueous acetone was added and sealed the flask with cotton plug covering with aluminum foil and kept in electrical shaker for 2 h for extraction. Then it was filtered through Whatman filter paper No. 1 and sample was kept in refrigerator at 4 °C until analysis.

ESTIMATION OF FLAVONOIDS:

Procedure: Quercetin was used to make the calibration curve.

Stock Solution of Extracts: 100 mg of the each extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol.

All prepared solutions were filtered through whatmann filter paper before measuring.

ESTIMATION OF REDUCING SUGAR:

Standard glucose solution : 0.1g anhydrous glucose was dissolved in distilled water and the volume was made up to 100 ml with distilled water. Series of standards using glucose (0–500 µg) were analysed and and graph plotted. Concentration of reducing sugars in extract was calculated from the standard curve.

QUALITATIVE AND QUANTATIVE

ANALYSIS ¹³OF GALLIC ACID BY HPTLC¹²:

Materials and Methods **HPTLC analysis** *Chemicals and standard gallic acid*

Gallic acid was obtained from Titan Biotech Ltd. and other reagents were used of analytical grade E-Merck. Silica gel 60 F ₂₅₄ precoated Thin Layer Chromatography (TLC) aluminium plate was used of E-Merck.

Chromatographic conditions

Chromatography was performed on precoated silica gel 60 F ₂₅₄ HPTLC plates (10.0 x 10.0 cm). Methanolic solutions of standard compound (gallic acid) and samples of known concentrations were applied to the plate positioned at 10 mm from the bottom and 19 mm from the side of the plate having 8 mm bandwidth using a CAMAG Linomat 5 automated TLC applicator with the nitrogen flow providing a delivery speed of 150 nl/s from the syringe.

Detection of Gallic acid

Plate was eluted in pre-saturated CAMAG twin trough glass tank with the mobile phase Toluene: Ethyl Acetate: Formic Acid: Methyl alcohol (6:6:1.6:0.4 v/v/v/v) to a distance of 86.2 mm at room temperature. After

**A COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDY OF ASHOKA
(*Saraca asoca* (Roxb.) de Wild) TWAKA SAMPLE FROM DIFFERENT DESHAS**

drying, the spots were visualized under CAMAG UV cabinet (254 and 280 nm). Then the plate was scanned using CAMAG TLC scanner 3 equipped with WINCATS software (CAMAG). The identification of gallic acid in methanolic solution of flower and leaf of *Saraca asoca* was confirmed by superimposing the UV spectra of samples and standards within the same retention factor (Rf) value.

Results :

HPTLC ANALYSIS OF GALLIC ACID

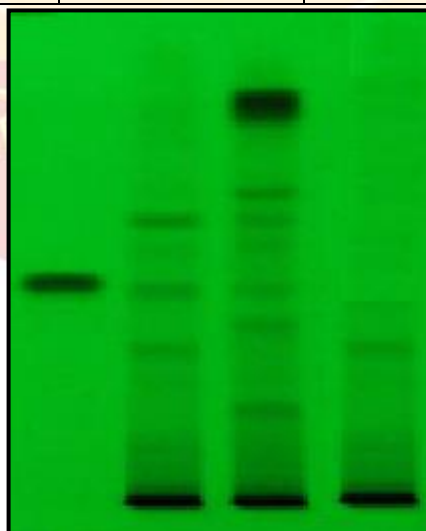
Result of HPTLC:

Photograph of chromatograms of the standard Gallic acid at 280 nm obtained in the petroleum ether

extract of dried bark of *Saraca asoca* are given in **Figure 1**. The Gallic acid bands in sample chromatogram of *Saraca* sp. are identified and confirmed by comparing the chromatogram obtained from the reference standard solution (**Figures 2**) by comparing retention factor (Rf) of Gallic acid from sample and standard solution. The Rf value of standard gallic acid is 0.44, whereas the Rf value of bark extracts of the *Saraca asoca* is 0.32, 0.42 and 0.27 which coincides with standard Rf value of gallic acid.

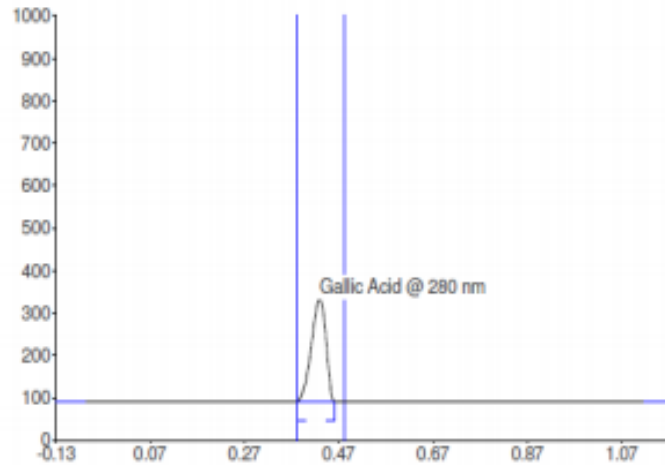
TABLE NO 31. SHOWING QUANTIFICATION OF GALLIC ACID:

	Standard gallic acid	Anup desha	Sadharan desha	Jangal desha
Rf value	0.44	0.42	0.32	0.27



GA S-2 S-1 S-3

Fig. 1: Photograph of chromatograms obtained at 280 nm from standard Gallic acid (1) Pet ether extract – Sample (2); Pet ether extract – sample 1 and Pet ether extract – sample 3 of *Saraca asoca*



Graph No.1: Chromatogram of Gallic Acid in the petroleum ether extract of bark of *Saraca asoca*

DISCUSSION

DISCUSSION OF HPTLC :

The HPTLC analysis of all three samples showed the higher concentration of Gallic Acid in Anup Desha sample and lowest Jangal Desha. The concentration of Gallic Acid in Anup, Sadharan and Jangal is 0.42, 0.31 and 0.27 respectively. The standard value of Gallic Acid is 0.44.

DISCUSSION OF RESULT :

On the basis of above discussion it is cleared that the sample from Anup Desha show higher concentration of active phyto constituents in compare to other sample i.e. Sadharan and Jangal desha.

By this we can tell that the place (Desha) from where the drug is

collected is very much important and importance to Desha is also given while collecting drug.

CONCLUSION

After Subjecting all the samples for through pharmacogonastic & Phytochemical investigation as per standard and comparing them in all parameters, following conclusion are drawn.

- The present analytical study discloses the fact that the quantity of Phytoconstituents, thickness of Bark, extractive values, varies in different places, to a large extent in Anup Desha.
- The Pharmcogonestical study shows that the parameters of all the sample

**A COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDY OF ASHOKA
(*Saraca asoca* (Roxb.) de Wild) TWAKA SAMPLE FROM DIFFERENT DESHAS**

are almost similar to the CCRAS standard values.

- The Major active principle like phenols, flavonoids, Tannins, reducing sugars, Gallic acid are present in higher concentration in Anup Desha and Sadharan & Jangal contain less concentration.
- Total Quantification of Gallic acid by HPTLC method is more in Anup Desha sample.
- All these criteria suggest to collect the Ashoka Bark from Anup Desh in order to increase, the Qualitative and Quantitative yield of the drug. So that the drug will possess maximum thereapeutic value.

List of References :

1. Agnivesa, Revised by Charaka & Dridhabala "CHARAKA SAMHITHA" EdiYadavji trikamji Commen: Chakrapanidatta, Ayurvedadipika, Publication, Chaukhamba Viswabharathi, Varanasi(UP) Reprint1 984 Vimanasthana, chap 8 verse 87 p.no 275
2. Vagbhataacharya "Ashtanga Hrudayam" 'Sarvangasundari' teeka by Arunadatta, Edited by Paradkar harishastri, Reprint 2000 Krishnadas Academy Varanasi; sutra sthana chap 12 verse 66,67 Pp 207
3. Sushruthaacharya "SUSRUTHA SAMHITA" Commen: Dalhanacharya Nibandhasangraha, Edin14, Chaukhamba Orientalia,Varanasi, sutrasthana Chap

37, Verse- 5 p.no 1599.

4. WHO Guidelines, Good agricultural and collection practices(GACP) for medicinal plants World Health Organization, Geneva 2003.

5. Ved. D.K, Goraya. G.S, "Demand And Supply Of Medicinal Of Plants In India", FRLHT 2008. P.139.

6. Sushruthaacharya "SUSRUTHA SAMHITA" Shastri.A. Ayurveda tatwa sandipika, Chaukhamba Sanskrit sansthan,Varanasi,2003 Edit.4, vol 1; chikitsa sthana Chap 4, Verse-32 p.no.28

7. Agnivesha,reviseby Charakand Dridabala"CharakaSamhita"Vidyotinitika, Kashinath shastri,Goraknath hindi commentary, Chaukhambha Bharati Academy,Varanasi,reprint2009,SutrasthanaChap4/47 p.no.66

8. Vagbhataacharya"AshtangaHrudayam" Sarvangasundari'teekabyArunadatta,Edited by paradkar harishastri, Reprint 2000 Krishnadas Academy Varanasi; Sutrasthana chap verse26P.no237

9. Bhavmishra, 'Bhavaprakash Nighantu' Singh. A. Chaukhamba Orientalia

Delhi 2007; Edit 1, chap 4pushpadivarga, verse 43 p.148.

10. Sharma P.C, Yelne M.B,Dennis T.J,Database on medicinal plants used in Ayurveda,CCRAS,New Delhi,2001,Vol-3 p .76.

11. Hanchinmane N R, Pharmacognostic and Phytochemical study of Ashoka (*saraca indica*) W.R.T different market samples, S.B.M.K. Ayurved

**A COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDY OF ASHOKA
(*Saraca asoca* (Roxb.) de Wild) TWAKA SAMPLE FROM DIFFERENT DESHAS**

Mahavidyalaya, Belgaum, 2008.

Dong.M.W, "Modern HPLC for Practicing Scientists" Wiley Interscience, USA 2006 P.no 2-13

12. Rathee. P. et al Quantitative analysis of (+)- catechin in Saraca asoca HPTLC, Dept. of Analytical chemistry, PDM College of Pharmacy, Bahadurgarh, Harayana, Dept. of Pharmacognosy and Phytochemistry, JCDM College of Pharmacy, sirsa, Harayana 2010.

13. Rathee. P. et al Quantitative analysis of (+)- catechin in Saraca asoca

HPTLC, Dept. of Analytical chemistry, PDM College of Pharmacy, Bahadurgarh, Harayana, Dept. of Pharmacognosy and Phytochemistry, JCDM College of Pharmacy, sirsa, Harayana 2010.

14. www.derpharmachemica.com

Corresponding author:

Dr. Uday Pratap Singh

Lecturer in Dravyaguna Shanti Ayurvedic Medical College and Hosptial Ballia UP

Email: dr.up85singh@gmail.com

Published BY:

Shri Prasanna Vitthala Education and Charitable Trust (Reg)

Source of Support: NIL

Conflict of Interest : None declared