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Research Article

Physico-chemical Standardization of *Butea Monosperma* (Lam.) Kuntze (Palasha): An Ayurvedic Drug

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ABSTRACT

The present communication attempts to investigate pharmacognostical and physicochemical analysis and chromatographic profiles of *Butea monosperma* (Lam.) Kuntze (fabaceae). Flowers are used as drug in many ailments like eye disease, chronic fever, enlargement of spleen, leucorrhoea, epilepsy, leprosy and gout etc. Identification of plants with botanical verifications is essential as adulteration due to misidentification of plant species or parts are common. Standardization of medicinal plant product is the prime need of the current time. The significant popularity of HPTLC in the analytical testing of pharmaceutical, bulk drugs and herbal lends its fame to the attributes. Therefore, HPTLC studies of alcoholic extract of *Butea monosperma* flowers were also carried out to lay down the fingerprint profile of drug. The study revealed specific identities for *Butea monosperma* (Lam.) Kuntze, which may play a key role in identification of plant and can be useful in standardization of the herbal drugs.

Key words: Butea monosperma, Flowes, Physico-chemical, HPTLC fingerprint.

INTRODUCTION

Medicinal plants have been used in virtually in all cultures as a source of medicine since times immemorial. Herbal medicine is still the mainstay of health care in several developing countries. The efficacy and safety of herbal products therefore rely on the quality and proper identification of the raw material or the original plant source. One major obstacle that might impair the potential use of traditional medicine as medicine of choice is the lack of standardization. Adulterations and substitutions are common in raw material trade of medicinal plants. Unintentional adulterations also exist in herbal raw material trade due to various reasons such as confusion in vernacular names between indigenous systems of medicine and local dialects, lack of knowledge about the authentic plant, non-availability of the authentic plant, similarity in morphology and /or aroma or careless collection.¹ To avoid this accurate authentication it is very important to prevent the adulteration of target plant with other plant species. Standardization of medicinal plant product is the prime need of the current time. Many of them do not have uniform standards and analytical procedures to justify their quality and purity. Modern techniques such as HPTLC, HPLC, and GC etc. can be used to develop the methods for the quantification of marker compounds in these types of multicomponent herbal formulations.

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Identification of plants with botanical verifications is essential as contamination due to misidentification of plant species or parts is common. Therefore, it becomes necessary to develop more effective, accurate, reliable and sensitive methods for the authentication of herbs. In the present study an effort has been made to establish physicochemical, and pharmacognostic, parameters which could be helpful in identification of the authentic plant samples and differentiating it from adulterants.³ Chromatography is a separation technique whereby the components of a mixture may be separated by allowing the sample to be transported through packed bed of material by fluid mobile phase. ⁴⁻⁵ Out of almost 700 pharmaceutical formulations that are documented in the USP almost 43% of the procedures are those documented by thin layer chromatography. The significant popularity of HPTLC in the analytical testing of pharmaceutical, bulk drugs and herbal lends its fame to the attributes. $^{6-7}$

MATERIALS AND METHODS

Material: Fresh flowers, (Fig.-1) were collected from different locations of Ghaziabad and dried in shade for 10 to 15 days. (Fig.-2)

The equipment used for HPTLC were A Cammag HPTLC system equipped with a sample applicator Linomat V, Automatic Multiple Developer- 2 Chamber, TLC Scanner 3, Reprostar 3 and Wincats an integrated Software 4.02 (Switzerland), and AR grade Chemicals alcohol, toluene, ethyl acetate, formic acid, potassium hydroxide, anisaldehyde, sulphuric acid, methanol used were obtained from S.D. Fine Chem. Ltd. (Mumbai, India). TLC Aluminium plate pre coated with silica gel60 GF_{254} (10X10 cm; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India).



Figure1: Close view of Flower



Figure2: Shade dried Flower

Methodology:

(a) **Physico-chemical studies:** Like total ash, acid insoluble ash, water soluble extractive, and pH of 5 % w/v solution of aqueous extract values were carried out as per the Ayurvedic Pharmacopoeia of India guidelines⁸.

(b) Preparation of extract for HPTLC: 1g of coarsely powdered drug sample was extracted with 100 ml alcohol for 24 hours by cold extraction method. The extracts were filtered by Whatmann no. 42 filter paper and make up to 100 ml in a volumetric flask. Filtrate was

concentrated to 10 ml and used for TLC.

(c) **HPTLC method:** TLC Aluminium plate pre coated with silica gel60 GF_{254} (10X10 cm; 0.2 mm thick) were used with Toluene: Ethyl acetate: Formic acid (5:4:1) as solvent system. Samples were spotted (7 micro litre and 9 micro litre) on TLC pre coated plate by using Linomate V applicator. Cammag Twin Through

Chamber (20X10 cm) with SS lid was used for development of TLC plates were developed upto 8 cm and plates air dried at room temp. TLC profiles were snapped by Cammag Reprostar 3 under UV Light 254 nm, and UV Light 366 nm then sprayed (derivatized) with Anisaldehyde- Sulphuric Acid reagent followed by heating at 105 °C for 10 minutes and after derivatization under UV Light 366 nm.

RESULTS AND OBSERVATION Botanical profiles

Butea monosperma (Lam.) is commonly known as flame of forest, belongs to the family fabaceae. An erect medium sized tree with somewhat crooked trunk, 12 - 15 m high with irregular branches, commonly found throughout the greater part of the country. Flowers large, in a rigid racemes 15 cm long, 3 flowers together form the tumid nodes of the dark olive-green velvety rhachis: pedicels about twice as long as the calyx, densely brown-velvety: bracts and bracteoles small deciduous. (Fig.-3)



Figure3: Rigid raceme

Physicochemical parameters

The percent of foreign matter, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, and pH of 5 % w/v solution of aqueous extract has been shown in Table 1.

Table 1: Physicochemical parameters of flower of				
Butea monosperma (Lam.) kuntze				
Parameters	Average Values			

1 al alletel s	Average values
Total ash	4.25 %
Acid-insoluble ash	0.63 %
Water soluble extractive	17.63 %
pH (5% w/v aqueous extract)	4.8 %
Alcohol soluble extractive	6.84 %

HPTLC study

The alcoholic extracts were used to carry out HPTLC. TLC of the drug on Silica Gel60 GF ₂₅₄ pre coated plate using Ethyle acetate: Methanol: Water (100:15:5) v/v as mobile phase shows, under UV Light 254 nm four spots appeared at Rf. 0.12, 0.17, 0.56 and 0.63 (all dark grey)

Table 2: HPTLC details of Sample solution of Butea monosperma, Flower				
S.No. of	Visualization		After Derivatization	
resolved bands	UV 254 nm	UV 366 nm	Visible range	UV 366 nm
1	0.12(dark grey)	0.12(reddish brown)	0.12(yellow)	0.12(dark brown)
2	0.17 (dark grey)	0.41(blue)	0.17(yellow)	0.17(dark brown)
3	0.56(dark grey)	0.53(greenish grey)	0.50(brown)	0.50(dark grey)
4	0.63(dark grey)	0.63(reddish brown)	0.56(yellow)	0.56(dark yellow)
5	-	0.78(red)	0.63(yellow)	0.63(dark yellow)
6	-	0.84(blue)	-	0.84(sky blue)
7	-	0.90(bright blue)	-	-

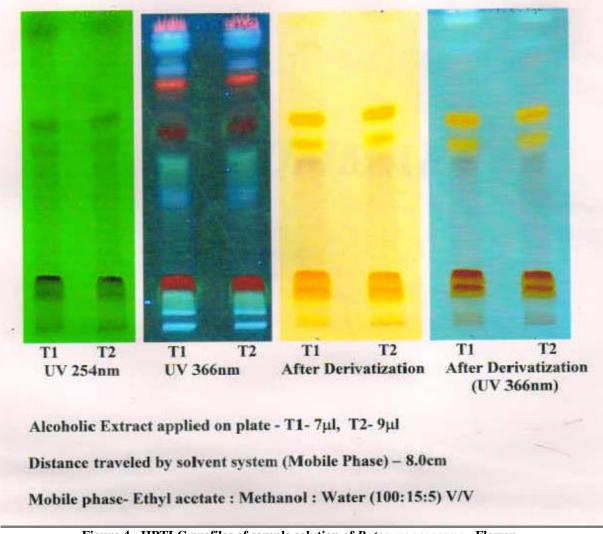


Figure 4 - HPTLC profiles of sample solution of Butea monosperma - Flower.

and under UV Light 366 nm seven spots appeared at Rf. 0.12 (reddish brown), 0.41 (blue), 0.53 (greenish gray) and 0.63 (reddish brown), 0.78 (red), 0.84 (blue) and 0.90 (bright blue). TLC plate was sprayed (derivatized) with Anisaldehyde- Sulphuric Acid reagent followed by heating at 105 0 C for 10 minutes; five spots appeared at Rf. 0.12, 0.17 (both yellow), 0.50 (brown), 0.56 and 0.63 (both yellow). After derivatization under UV Light 366 nm six spots appeared at Rf. 0.12, 0.17 (dark brown), 0.50 (dark grey), 0.56, 0.63 (dark yellow) and 0.84 (sky blue). Data also shown in Table 2.

DISCUSSION

This study presents a set of diagnostic characters of *Butea monosperma* (Lam.) Kuntze that will help to identify the drug in fragmentary condition as well as in whole form. The results of parameters for preliminary physiochemical screening, UV analysis and HPTLC studies can act as biomarkers for identification and authentification of raw drug samples and play an important role in quality control and prevention of adulteration.

REFERENCES

- 1. Mitra S.K. and Kannan R. (2007) A Note on Unintentional Adulterations in Ayurvedic Herbs. *Ethnobotanical Leaflets*; 11: 11-15.
- 2. Jeganathan N.S. and Kannan K. (2008) Indian J.Pharm.Educ.Res. 42(1); 59-64.
- 3. Gupta etal (2009) Pharmacopoeial Standardization of *Hibiscus rosa sinensis* Linn. *International Journal of Pharmaceutical and Clinical Research*; 1(3): 124-126.
- Szepesi, Gazdag M. and Mihalyfi K., Selection of HPLC methods in pharmaceutical analysis -III method validation, www.labcompliance.com Page 21 *J.Chromatogr.* 464, 265-278.
- 5. Zaltkis. A. and Kaiser. R. E. (1977), HPTLC- High Performance Thin Layer Chromatography, 619-625A.
- 6. Sethi P. D. (1997), Quantitative Analysis of Drugs in Pharmaceutical Formulations, Unique Publisher, 11.
- J.M.Green, (1995), a Practical Guide to Analytical Method Validation, Anal.Chem. News & Features, May 1, 1996, 305A/309A.
- 8. The Ayurvedic Pharmacopoeia of India, Part- I, Volume- I, First Editio