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The fingerprinting of extracts of flowers leaves, bark and seeds of Indian dhak tree, *Butea monosperma* by HPTLC technique.

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Abstract: Flame of forest, Indian dhak tree *Butea monosperma* popularly known as palash is the integral part of traditional system of medicine. Bark, leaf, seed and flower part of *Butea monosperma* are used traditionally in treating various ailments. Hence the comparative study of bark, leaf, flower and seed was undertaken to study macroscopy, microscopy. Also phytochemical screening was carried out using HPTLC technique. Also the various solvent systems were studied to optimize the separation of various phytoconstituents present and to develop chemoprofile.

Key words: *Butea monosperma*, HPTLC, Authentification and identification, fingerprinting

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I. INTRODUCTION

Indian Dhak tree *Butea monosperma* (Flame of forest) popularly known as Palash has been widely used in the traditional Indian medical system of 'Ayurveda' for the treatment of a variety of ailments. The various parts of tree like bark,seeds, flower, leaves have shown the various therapeutic effects like antihyperglycemic, hepatoprotective, antioxidant, anti-inflammatory, Chemopreventive and anti-cancer activity. [1-7]

Butea monosperma is a deciduous, erect, medium size tree. The flowers of Butea monosperma are bright orange in colour, densely clustered, odourless and velvety in touch. The February to March is the flowering season. The bark is brown in colour and breaks with laminated fracture in outer part and fibrous in inner part. Leaves are alternate, trifoliate. Leaves has entire margin and pinnately trifoliate. The seeds are flat and compressed, oval and have smooth surface. Pods are silky and much compressed tomentose 10-13 cm long containing one seed at its apex.

Present work is undertaken to study the HPTLC fingerprinting of extracts of flower, bark, leaf and seed.is carried out using HPTLC fingerprinting technique. [8]

II. MATERIALS AND METHOD

Procurement, Identification and Preparation of sample

All parts flowers, bark and leaves of the Palash tree were collected from the forest area of India (Kasara, Maharashtra). The seeds of *Butea monosperma* plants were collected from the local market of Mumbai, India. The collected plant materials were cleaned. The authentication of the crude drug materials flowers, bark, leaves and seeds were carried out at Blatter Herbarium, St.Xavier's College, Mumbai, India. Plant materials flowers, bark, leaves and seeds were dried at 50^0c ($\pm$ 1^0 c) and then powdered in the mixer.

The Fingerprinting was carried by High performance thin layer chromatography (HPTLC) method

The comparative phytochemical screening was carried out using standard method as recommended by USP. A qualitative HPTLC phytochemical screening and HPTLC fingerprinting was performed on bark, leaf, seed and flower extracts prepared. The characteristic fingerprint profile developed may be used for authentication, identification, standardization and quality evaluation. The present study was undertaken to develop chemoprofiles of the extracts of dried bark, leaf, seed and flower of *Butea* monosperma.[10-13]

Preparation of sample for fingerprinting analysis

Each powered drug of flower, bark, leaf and seed of *Butea monosperma* weighed about 15 g and methanolic extract was prepared using soxhlet extraction method. The prepared extract was used for phytochemical screening and fingerprinting.

For the phytochemical screening each extract in quantity 5 µl, 10µl, 15 µl was spotted on pre coated TLC Aluminium plates silica gel 60F 254 (Merck) with CAMAG Linomat V applicator. The plate was developed in glass twin trough chamber (20 cm x 10cm) presaturated with mobile phase. The mobile phases and derivatising reagents for respective phytochemical is mentioned in Table. The plates were derivatized and scanned using TLC Scanner 3(CAMAG).

2.6 HPTLC Fingerprinting

Similarly HPTLC fingerprinting carried out using methanolic extract prepared as stated. For chemoprofile bark extract in quantity 10 µl, 15 µl, 20 µl and leaf, seed, flower extracts in quantity 1 µl,2 µl, 3 µl were spotted on pre coated TLC Aluminium plates silica gel 60F 254 (Merck) with CAMAG Linomat V applicator. The plate was developed in glass twin trough chamber (20 cm x 10cm) presaturated with mobile phase. The mobile phases studied for separation have mentioned in the table number 6. The plates were derivatized and scanned using TLC Scanner 3 (CAMAG).

The mobile phases and derivatizing agents for chemoprofile development of the methanolic extract of bark, leaf, flower, seed of *Butea monosperma*

Sr. No	Mobile Phase	Derivatizing agent
A	Toluene: Chloroform: Methanol (4:4:1 v/v/v)	Anisaldehyde sulphuric acid reagent
B	Toluene : Ethyl acetate: Diethylamine (7:2:1 v/v/v)	Anisaldehyde sulphuric acid reagent
C	Dichloromethane: Methanol: Acetic acid (17:1.6:1.2 v/v/v)	2-aminoethyldiphenyl borinate in methanol

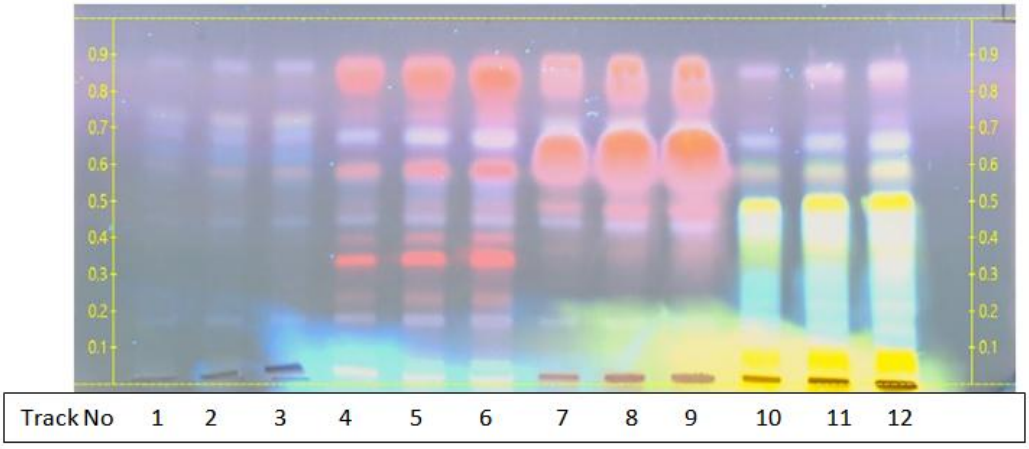
3.4 HPTLC fingerprinting

Phytochemical studies using HPTLC method have shown the presence of alkaloids in leaf extract. The bitter principles were found to be present in the extracts of leaf, seed, and flower. The essential oils were found to be present in bark, leaf, seed, and flower. The flavonoids were found to be present in leaf, seed, and flower. Also leaf and flower extracts have shown the presence of glycosides. Also saponins were found to be present in leaf and seed extracts. Tannins found to be present in all extracts of bark, leaf, seed, and flower. Whereas triterpenoids were found to be present in extract of leaf, seed, and flower. The number of spots and respective Rf values of respective phytochemicals are recorded in Table number 11

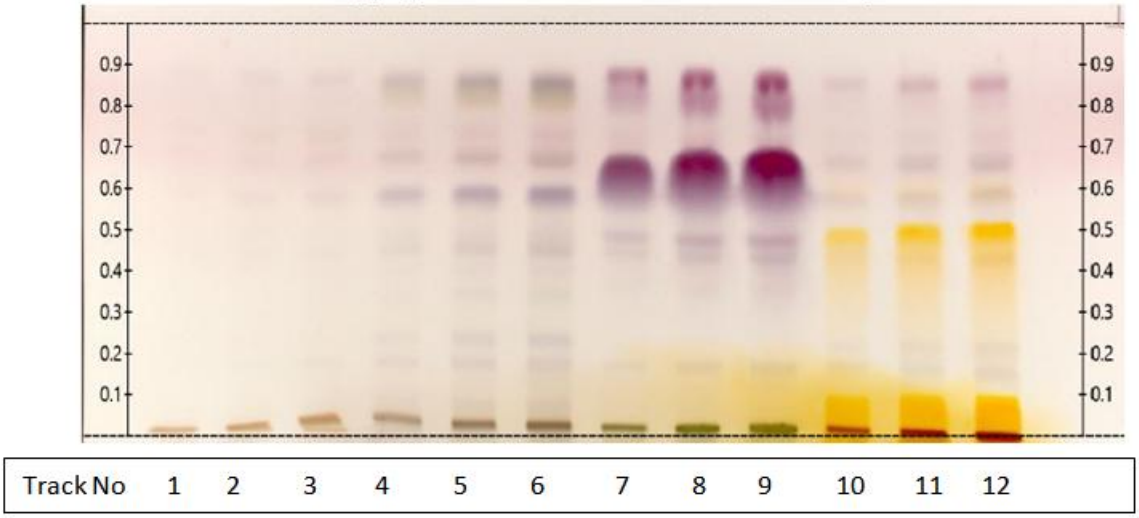
HPTLC Fingerprint

HPTLC fingerprinting studies of methanolic extract showed distinct band pattern before and after spraying with derivatizing reagent. Solvent system A [Toluene:Chloroform: Methanol (4:4:1 v/v/v)] was found to be more suitable and have better separation of compounds than the other two solvent systems B and C [Toluene : Ethyl acetate: Diethylamine (7:2:1 v/v/v)] and [Dichloromethane: Methanol: Acetic acid (17:1.6:1.2 v/v/v)] Solvent systemB was found to give dragging effect whereas solvent system C showed the less separation of phytoconstituents.

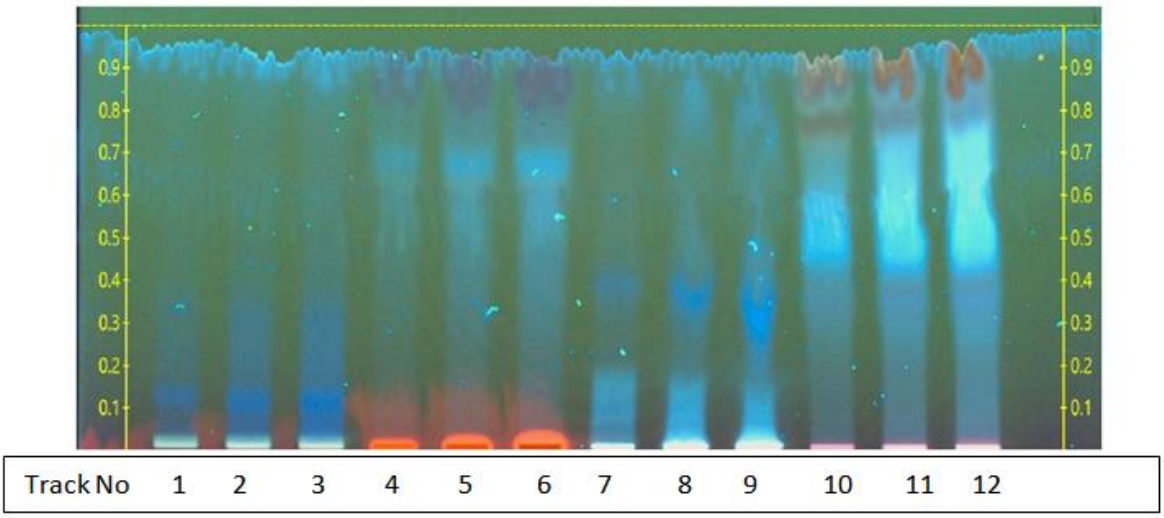
The HPTLC fingerprinting for the extracts of bark, leaf, seed and flower of *Butea monosperma* shows the dragging effect at 366nm with solvent system B



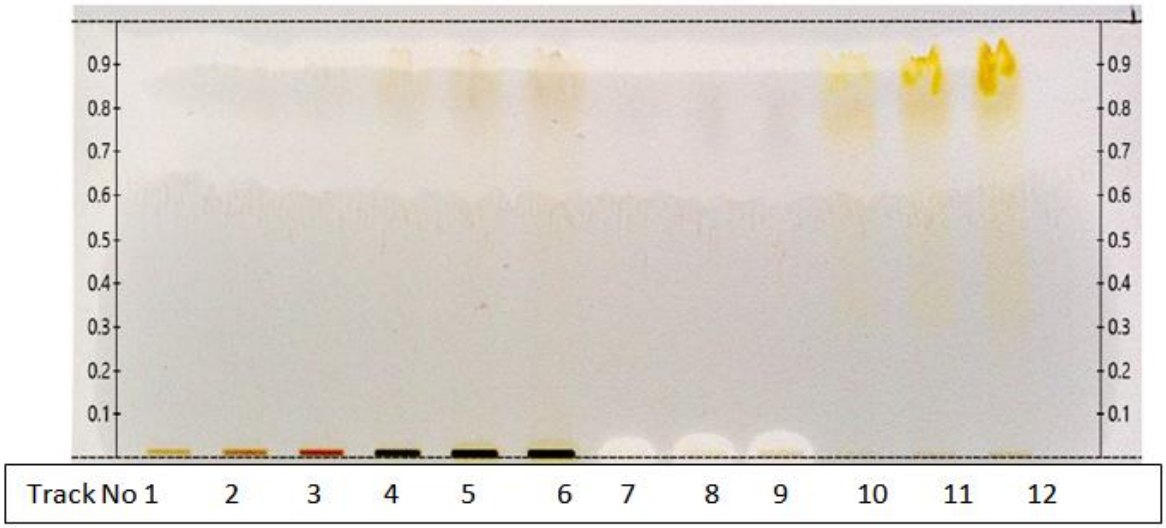
The HPTLC fingerprinting for the extracts of bark, leaf, seed and flower of *Butea monosperma* shows the dragging effect with solvent system B at visible.



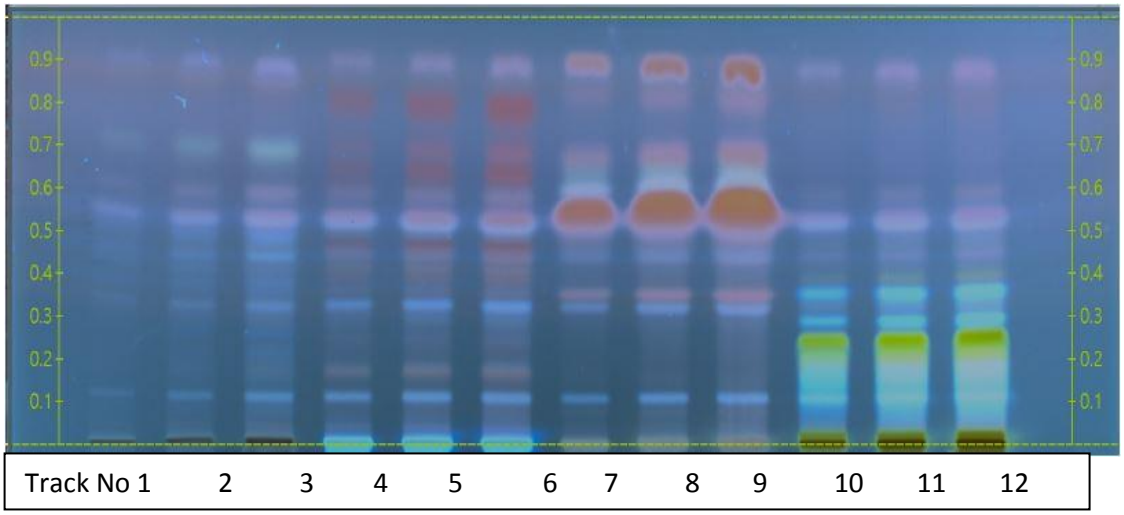
The HPTLC fingerprinting for the extracts of bark, leaf, seed and flower of *Butea monosperm a* showed the less separation at 366nm with solvent system B



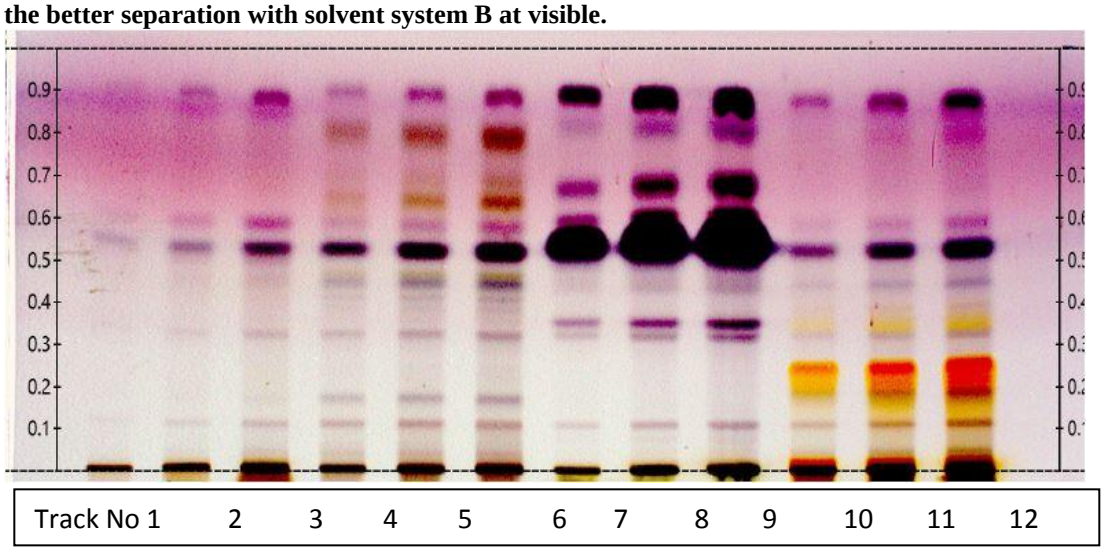
The HPTLC fingerprinting for the extracts of bark, leaf, seed and flower of *Butea monosperma* showed the less separation with solvent system B at visible.



The HPTLC fingerprinting for the extracts of bark, leaf, seed and flower of *Butea monosperma* shows the better separation at 366nm with solvent system B



The HPTLC fingerprinting for the extracts of bark, leaf, seed and flower of *Butea monosperma* showed the better separation with solvent system B at visible.



III. DISCUSSION

The check for quality and standardization of medicinal plants of therapeutic potential is important to maintain the quality of the herbal products. The macroscopy and microscopy study is an integral part of a standardization and identification. The results obtained from the present study may serve as a basis for identification, collection and investigation of plant.

Phytochemical studies using HPTLC method have shown the presence of alkaloids, bitter principles, flavonoids, triterpenoids, glycosides and saponins in leaf extract. The seed extract showed the presence of bitter principle, flavonoids, saponnins and tritepenoids. The flower extract showed the presence of bitter principles, glycosides, flavonoids and triterpenoids. The essential oils and tannins were present in all extracts of bark, leaf, seed, and flower. The phytochemical screening may help in identification and isolation of phytoconstituents.

The chemoprofile developed with various solvent system using HPTLC can give idea about various phytoconstituents present and the HPTLC fingerprint profile respective Rf values can be used for confirming identity and purity of a plant and can also used for further research on the medicinal properties of plant.

IV. CONCLUSION

The demand for the medicinal plants and plant product is on rampage. Also the commercialization in production has created the need for standardization. The setting the standards and quality for medicinal plants and plant based products has a different approach than that of the synthetic drugs.

Phytochemical screening and HPTLC chemoprofile gives information about various phyoconstituents presnt and this information can serve as base for separation and isolation of phytoconstituents even in identification and authentication. These methods can also useful in determination of presence of pesticides and toxins in medicinal plants. Indian dhak tree, flame of forest *Butea monosperma* commonly known as Palash known for its various medicinal properties. Hence the present study can serve as a base for identification, authentification and standardization.

Conflicts of Interest
All authors have none to declare.

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