

**COMPARATIVE PHYTOCHEMICAL STUDY OF STEM BARK
VERSUS SMALL BRANCHES OF *BUTEA MONOSPERMA* LAM.
USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC
ULTRA VIOLET DETECTION METHOD**

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ABSTRACT

Butea monosperma Lam. belongs to family Fabaceae, popularly known as 'dhak' or 'palas'. *B. monosperma* is extensively used in Ayurveda, Unani and Homeopathic medicine. The plant *B. monosperma* is known to possess numerous medicinal properties and almost all parts of the plants have been used since decades in medicines and for other purposes. The bark have been reported for various pharmacological properties includes anthelmintic, anticonceptive, anticonvulsive, antidiabetic, antidiarrhoeal, antiestrogenic, antifertility, antimicrobial, antifungal, antibacterial, anti stress, chemo preventive, haemagglutinating, hepatoprotective, radical scavenging, thyroid inhibitory, anti per oxidative, hypoglycaemic effects and wound healing activities. The unique patterns of the chromatographic fingerprint were validated by analyzing stem bark and small branches

of *B. monosperma*. Our results revealed that the chromatographic fingerprint combined with similarity measurement could efficiently identify and distinguish *B. monosperma* stem bark and small branches. The phytochemical fingerprint profiling of stem bark and small branches of *B. monosperma* were found similar as an official part of *B. monosperma* plant i.e. stem bark, therefore small branches may be used in place of stem bark and vice-versa after comparison and confirmation of same pharmacological activities. The method can also be used for identification of different *B. monosperma* species and adulterants.

KEYWORDS: *Butea monosperma* Lam., HPTLC–UV detection, phytochemical fingerprint profiling analysis.

Abbreviations: HPTLC–UV, high performance thin layer chromatography-ultra violet detection; R_f , retention factor; **min.**, minutes; **St. Bk.**, stem bark., **Sm. Br.**, small branches;

INTRODUCTION

Butea monosperma Lam. [Fig.1] belongs to family Fabaceae, which comprises of 630 genera and 18,000 species. The plant is popularly known as 'dhak' or 'palas' and commonly known as flame of the forest, palash, mutthuga, bijasneha, khakara, chichara, Bastard teak and Bengal kino etc. It is a wild, medium sized tree found throughout the deciduous forests and also in open areas.^[1-3] The tree is growing in abundance in most part of India, Berma, Srilanka, Pakistan, Bangladesh, Nepal, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia, and Western Indonesia. It is also common in some of the sandy area in Gujarat and Saurashtra. Nearer Bombay, the tree is common on the hills from Kalyan to Igatpuri or to Khandala along the main road or the railway lines.^[4-5] It is considered as one of the most beautiful trees of India due to its gorgeous canopy of scarlet flowers which looks like a flame.^[6] It is a slow growing tree that reaches a height of 40 to 50 feet.^[7] *B. monosperma* is extensively used in Ayurveda, Unani and Homeopathic medicine and has become a cynosure of modern medicine. The plant is commonly used by the rural and tribal people in therapeutic uses. The plant *B. monosperma* is known to possess various medicinal properties and almost all parts of the plants have been used since decades in medicines and for other purposes.^[8] The tree provides wood, gum and dye. Wood is used to make well curbs and water scoops. The wood pulp is useful in newsprint manufacturing. Gums are used in leather industry, drugs and in some food preparations.^[7] It is considered to be the purest of all woods and is used in the religious rituals especially during marriage and cremation of the dead body by Hindus.^[9] This plant species has been found to display a wide variety of biological activities. The plant is traditionally reported to possess astringent, bitter, alterative, aphrodisiac, anthelmintic, antibacterial and anti-asthmatic properties.^[3] The plant *B. monosperma* is known to possess numerous medicinal properties and almost all parts of the plants have been used since decades in medicines and for other purposes.^[8] Roots are useful in filariasis, night blindness, helminthiasis, piles, ulcer and tumours. It is reported to possess antifertility, aphrodisiac and analgesic activities.^[10] It is also used in elephantiasis, impotency, snake bite, can causes temporary sterility in women and is applied in sprue and dropsy.^[3] It is used to heal the boil or sore in eye. Extract of the root is prepared by the process of distillation and is put in the eye (2 to 3 Drops) twice a day.^[11] One teaspoonful of root bark juice can be given orally a day for three days as contraceptive.^[12] Bark is fibrous, ash coloured and yield red

juice known as 'Butea gum' or 'Bengal kino'. It reported pharmacological properties include anthelmintic, anticonceptive, anticonvulsive, antidiabetic, antidiarrhoeal, antiestrogenic, antifertility, antimicrobial, antifungal, antibacterial, anti stress, chemo preventive, haemagglutinating, hepatoprotective, radical scavenging, thyroid inhibitory, anti peroxidative, hypoglycaemic effects and wound healing activities. It is considered valuable by druggists because of its astringent qualities and by leather workers because of its tannin and also is given in many forms of chronic diarrhoea. It has been used as a natural insecticide against houseflies and dried 'Moduga' flowers for preventing sunstrokes.^[3, 10, 11, 13] The stem region exuded the juice which get hardens into ruby coloured gum ('kamarkas') similar to 'kino'. This gum is used as a remedy for pain in waist. It is very nutritious and is used against loose-motions and healing the wound.^[9] The powder of the stem bark is used to apply on injury caused due to an axe, the juice of the stem is applied on goitre of human beings and the paste of the stem bark is applied in case of body swellings.^[11] Stem bark extract with jeera powder used for leucorrhoea, jaundice and skin diseases. The decoction of stem bark is said to be given as a tonic to women after child-birth.^[12] Leaves of *B. monosperma* are compound, with three leaflets, large and stipulate 10-15 cm long petiole. Leaflets are obtuse, glabrous above finely silky and conspicuously reticulately veined beneath with cunnate or deltoid base.^[3] The leaves are used in the preparation of 'pattal' (plates) considered to be pure and which are used particularly in religious ceremonies.^[9] They are also used for making cubs, bowls and beedi wrappers.^[8] The leaves are also used as appetizer, expectorant, astringent, anti-inflammatory, anodyne, aphrodisiac, in pimples boils, flatulence, colic worm infections, inflammations, arthralgia, haemorrhoids and night blindness.^[8,10] The flowers start appearing in February and stay on nearly up to the end of April. The size is nearly 2 to 4 cm in diameter. These tend to be densely crowded on leaflet branches. The flowers on the upper portion of the tree form the appearance of a flame from a distance.^[3] Flowers are a particular interest from a medicinal point of view as an anti-diabetic, anti-asthmatic, anti-inflammatory, antimicrobial, antibacterial, antifungal activity, anticonceptive, Hepatoprotective and also used in the treatment of leprosy, gout, diarrhoea, wound healing, diuretic and leucorrhoea.^[14] Flowers yields a brilliant yellow colouring matter due to presence chalcones.^[3, 15] Decoction of flowers is used to keep the white-ants away from the fields. The paste prepared from dried flowers is applied on the stomach which gives relief from stomach ache, urine problem and stone.^[9] In Sanskrit the flower is extensively used as a symbol of the arrival of spring and the colour of love. They are attracted by the smell and colour of the flower.^[13] The seeds of *B. monosperma* increase the semen.^[9] Seeds have anthelmintic property especially for

roundworms and tapeworms.^[3, 15] It is reported that the ethanolic extract of seeds of *B. monosperma*, on oral administration showed antifertility activity in mice and rats.^[11] The whole plant is used for timber, resin, fodder, medicine, and dye.^[13] It finds use both medicinally and commercially with each part of the plant having utility. Such herbal medicines may provide potential effect as of compared to the conventional available synthetic drugs with less or no side effects.^[3]

Chemical screening of the parts of the species has shown the presence of flavonoids, chalcones, linoleic acid and unsaturated fatty acids.^[12] Plant contains cantharic acid, proanthocyanidins, L- β -Phenylalanine derivatives, aleuritic acid, palasitricin, and major glycosides as Butrin, alanine, allophanic acid, butolic acid, cyanidin, histidine, lupenone, lupeol, (-)-medicarpin, miroestrol, palasimide and shellolic acid.^[16] Roots contain glucose, glycine, a glycoside and an aromatic hydroxy compound.^[17] The euphane triterpenoid 3 α -hydroxyeuph-25-ene and the alcohol 2, 14 dihydroxy-11, 12-dimethyl-8-oxo-octadec-11-enylcyclohexane,^[15] 3-Z-hydroxyeuph-25-ene, Stigmasterol-e-D-glucopyranoside and nonacosanoic acid has been reported in the stem.^[18] Stem bark containing β -sitosterol, kinnic acid, gallic acid, pyrocatechin and stigmasterol.^[18, 19] The other constituents reported in stem bark are Cajanin, isoformononetin; Stigmasterol; Butin; two known flavonoids, isobutrin (3, 4, 2', 4'-tetrahydroxychalcone-3, 4'-diglucoside) and the less active butrin (7, 3', 4'-trihydroxyflavanone-7, 3'-diglucoside); free sugars and free amino acids and (-)-3-hydroxy-9-methoxypterocarpan (medicarpin).^[15] The leaves contains Glucoside, 3, 9-dimethoxypterocarpan reported in ethyl acetate fraction of methanol extractives of leaves and hexane fraction of methanol extractives yielded 3- α hydroxyeuph- 25-enylheptacosanoate,^[18] Flavonoids, chalcones, tannins.^[20] The phytoconstituents reported in the flower are triterpene, butein, butin, isobutrin, coreopsin, isocoreopsin (butin 7-glucoside), sulphurein, isomonospermoside, chalcones, auronones, flavonoids (palasitricin, prunetin) 'chalkiness', aureoles, flavonoids (palasitricin, prunetin) and steroids.^[16, 18] The compounds reported in seed are plasmatic, linoleic acid, α -anryrin, β -sitosterol its glucoside and sucrose,^[21] 8-lactone of *n*-heneicosanoic acid, anti helminthic principle compound palasonin and its L- β -phenylalanine derivative.^[17] Oil (yellow, tasteless), proteolytic, lypolytic enzymes, plant proteinase and polypeptidase (similar to yeast tripsin), a nitrogenous acidic compound and monospermoside (butein 3-e-D-glucoside).^[16] From seed coat allophanic acid has been isolated and identified.^[18] Gum contains tannins, mucilaginous material and pyrocatechin.^[16] Resin contains the acid esters jalaric esters I, II and laccijalaric esters I, II,

III, IV.^[16, 17] Sap contains chalcones, butein, butin, colourless isomeric flavanone and its glucosides, butrin.^[16] The Imide palasimide has been isolated from the pods of this plant species.^[15]



Figure 1: *Butea monosperma*



Figure 2: Stem Bark.



Figure 3: Small Branches.

Botanical classification^[15]

Kingdom	Plantae – Plants
Sub-kingdom	Tracheobionta – Vascular plants
Super-division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Fabales
Family	Fabaceae – Pea family
Genus	<i>Butea</i> Roxb.ex Wild. – Butea
Species	<i>monosperma</i> (Lam.)

Formulations

Herbal Hair Loss Cream.^[22]

MATERIALS AND METHODS

Plant Materials and Chemicals

Stem barks [Fig. 2] and small branches of stem [Fig.3] of *B. monosperma* were collected in December 2013 and authenticated by Dr. R. K. Tiwari, Research Officer, Pharmacognosy, National Veterinary Research Institute & Hospital, Lucknow. All chemicals (AR grade) and TLC plates were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

Sample preparation

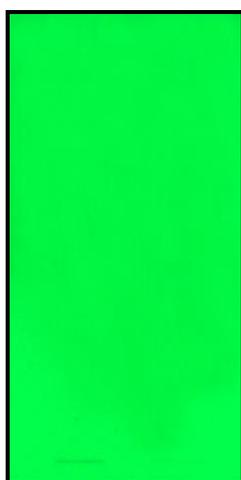
The plant parts were dried under a gentle stream of air in the laboratory till no loss in weight (temperature $30 \pm 2^{\circ}\text{C}$ and relative humidity $50 \pm 5\%$) and powdered in an electric grinder.

Conventional extraction of stem bark and small branches of stem of *B. monosperma* were performed at room temperature ($28^{\circ} \pm 3^{\circ}\text{C}$) with a variety of solvents ranging from non-polar to polar ones, i.e. *n*-hexane, ethyl acetate and ethanol. Dried and powdered parts of *B. monosperma* (10 g each) were extracted three times (3×50 mL) for 18 h of each extraction with each of the above-mentioned solvents separately. Each extract was filtered by using Whatman filter paper no. 1 and the solvents were removed under vacuum at 50°C , separately and concentrated up to 10 mL to get the sample solution of 100 mg mL^{-1} . 5 μL of each sample was applied separately to TLC plate for the development of fingerprints.

HPTLC-UV detection Method

High Performance Thin Layer Chromatography was performed on $10 \text{ cm} \times 10 \text{ cm}$ TLC plates pre-coated with $0.25 \mu\text{m}$ thin layers of silica gel 60 F₂₅₄ (E. Merck). Both samples (stem bark and small branches) were applied on the plates as bands 10 mm wide by use of a Linomat-IV applicator (CAMAG, Switzerland) fitted with a 100 μL syringe (Hamilton, Switzerland). The application positions X and Y were both 10 mm, to avoid edge effects. Linear ascending development to a distance of 80 mm with *Toluene: Ethyl acetate: 9: 1 (v/v)* and as mobile phase for both *n*-hexane extract was performed in a twin-trough glass chamber ($20 \text{ cm} \times 10 \text{ cm}$) previously saturated with vapours of mobile phase for 20 min. The plates were dried in air and visualized under $\lambda 254 \text{ nm}$ and $\lambda 366 \text{ nm}$ for ultra violet detection and taken the fingerprints as evident in Figures 4-5. Further, the same TLC plate was derivatized with anisaldehyde-sulphuric acid reagent and visualized in white light obtained fingerprints were as evident in Figures 6 using CAMAG Reprostar and WinCATs software (V1.4.2; CAMAG). HPTLC of ethyl acetate extract and alcoholic extract of both drugs was performed same procedure with the mobile phases of *Toluene: Ethyl acetate 7: 3 (v/v)* and *Toluene: Ethyl*

acetate 6:4 (v/v) respectively and then visualized in λ 254 nm, λ 366 nm and white light using CAMAG Reprostar and WinCAT's software as shown in Figure 7-12.



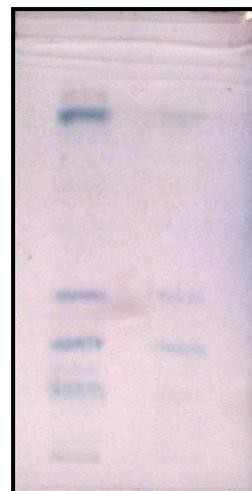
1 2
254 nm

Figure 4



1 2
366 nm

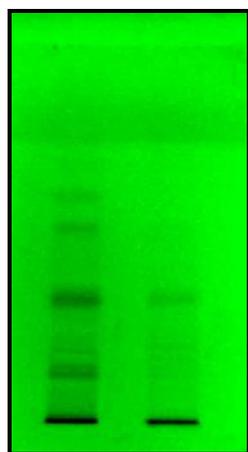
Figure 5



1 2
After derivatization

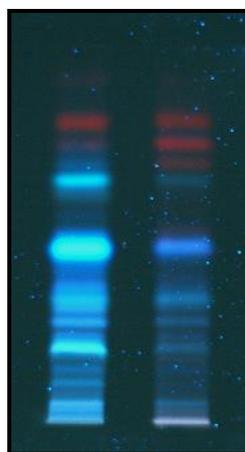
Figure 6

Figure 4-6: TLC fingerprint of *n*-hexane extract of *B. monosperma* (1= St. Bk.; 2= Sm. Br.)



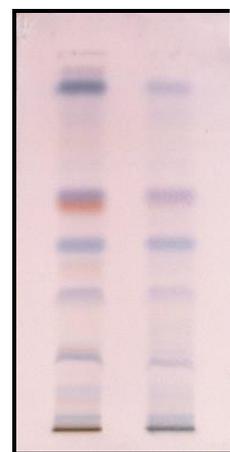
1 2
254 nm

Figure 7



1 2
366 nm

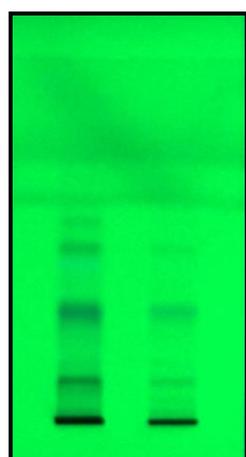
Figure 8



1 2
After derivatization

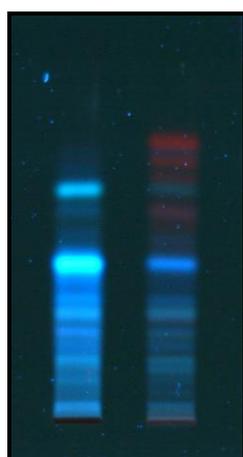
Figures 9

Figure 7-9: TLC fingerprint of ethyl acetate extract of *B. monosperma* (1= St. Bk.; 2= Sm. Br.)



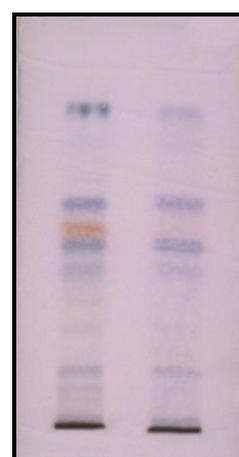
1 2
254 nm

Figure 10



1 2
366 nm

Figure 11



1 2

After derivatization with
Anisaldehyde sulphuric acid reagent

Figures 12

Figure 10-12: TLC fingerprint of ethanol extract of *B. monosperma* (1= St. Bk.; 2= Sm. Br.)

Table 1: R_f value of phytochemicals present in *n*-hexane, ethyl acetate and ethanol extract of *B. monosperma* (St. Bk. and Sm. Br.) at different wave-lengths.

Wave-length	<i>n</i> - Hexane extract		Ethyl acetate extract		Ethanol extract	
	Stem bark	Small branches	Stem bark	Small branches	Stem bark	Small branches
254	-	-	0.13, 0.15, 0.35, 0.51, 0.60	0.35	0.12, 0.31, 0.48, 0.55	0.12, 0.31, 0.48
366	0.09, 0.20, 0.34, 0.40, 0.49, 0.72	0.34, 0.40, 0.49, 0.72	0.07, 0.12, 0.15, 0.21, 0.28, 0.31, 0.35, 0.38, 0.47, 0.66, 0.70, 0.76, 0.84	0.07, 0.21, 0.28, 0.35, 0.47, 0.66, 0.71, 0.76, 0.84	0.13, 0.18, 0.22, 0.26, 0.30, 0.34, 0.43, 0.51, 0.59, 0.64, 0.70	0.15, 0.18, 0.22, 0.26, 0.30, 0.34, 0.43, 0.51, 0.58, 0.64, 0.66, 0.71, 0.77
Visible light after derivatization	0.18, 0.23, 0.29, 0.41, 0.88, 0.93	0.29, 0.41, 0.88	0.08, 0.12, 0.19, 0.21, 0.36, 0.41, 0.48, 0.59, 0.62, 0.91, 0.95	0.12, 0.19, 0.36, 0.48, 0.59, 0.62, 0.91	0.09, 0.13, 0.17, 0.29, 0.45, 0.51, 0.55, 0.63, 0.85	0.17, 0.29, 0.45, 0.51, 0.63, 0.85

RESULTS AND DISCUSSION

As per my knowledge no such study was found in literature for comparative phytochemical study of stem bark versus small branches of *B. monosperma* Lam. by using High

Performance Thin Layer Chromatographic-Ultra Violet detection Method. Comparative study of TLC fingerprints of stem bark and small branches of *B. monosperma* revealed that many similarities in phytochemical fingerprints were found and evident in Table-1 and Fig. 4-12.

Phytochemical fingerprints of *n*-hexane extract of stem bark and small branches showed no band under UV detection at 254 nm. Under 366 nm UV detection, stem bark and small branches showed six and four bands respectively, out of which four bands at R_f 0.34 (red) 0.40 (light blue), 0.49 (red) and 0.72 (blue) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, stem bark and small branches both were showed six and three bands respectively, out of which three bands at R_f 0.29 (blue), 0.41 (violet) and 0.88 (blue) were found similar as represented in Table 1 and Fig. 4-6.

Phytochemical fingerprints of ethyl acetate extract of stem bark and small branches under 254 nm represented five and one bands respectively, out of which, one band was found similar at R_f 0.35 (black). Under 366 nm UV detection, stem bark and small branches showed thirteen and nine bands respectively, out of which eight bands at R_f 0.07 (blue), 0.21 (blue), 0.28 (blue), 0.35 (blue), 0.47 (blue), 0.66 (blue), 0.76 (red) and 0.84 (red) were found similar. After derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light eleven and seven bands were visible in stem bark and small branches respectively out of which, seven bands at R_f 0.12 (blue), 0.19 (blue), 0.36 (dark blue), 0.48 (blue), 0.59 (orange) 0.62 (blue) and 0.91 (violet) were found similar as showed in Table 1 and Fig. 5-8.

Phytochemical fingerprints of ethanolic extract of stem bark and small branches under UV detection at 254 nm, showed four and three bands respectively, out of which three bands were found similar at R_f 0.12, 0.31 and 0.48 (All were black). While under 366 nm UV detection, stem bark and small branches showed eleven and thirteen bands respectively, out of which seven bands at R_f 0.22, 0.26, 0.30, 0.34, 0.43 and 0.64 (All were blue) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, stem bark and small branches showed nine and six bands respectively, out of which six bands at R_f 0.17 (brown), 0.29 (blue), 0.45 (blue), 0.51 (blue) 0.63 (blue) and 0.85 (blue) were found similar in both parts (St. Bk. and Sm. Br.) as evident in Table 1 and Fig. 10-12.

CONCLUSION

Phytochemical fingerprint profiling of various parts of *B. monosperma* indicated that different types of phytoconstituents present in each part but many similarities in fingerprinting were found in stem bark and small branches. The phytochemical fingerprint profiling of small branches of *B. monosperma* were slightly similar with stem bark as a official part of *B. monosperma* plant, therefore small branches may be used in place of stem bark and vice-versa after comparison and confirmation of same pharmacological activities. The R_f helped in evaluation of phytochemical diversity in different parts of *B. monosperma*. The phytochemical diversity was found more in stem bark followed by small branches at one geographical region. TLC phytochemical fingerprint profiling of *n*-hexane, ethyl acetate, ethanolic extracts of stem bark and small branches of *B. monosperma* have been given an idea about the presence of various phytochemicals in their reported parts. The TLC spots provided valuable clue regarding presence or absence of various phytochemicals or metabolites of the plants.

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