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


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, anti diarrhoeal, 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 compromises 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 filariosis, 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.\cite{11} One teaspoonful of root bark juice can be given orally a day for three days as contraceptive.\cite{12} Bark is fibrous, ash coloured and yield red juice known as ‘Butea gum’ or ‘Bengal kino’. It reported pharmacological properties include anthelmintic, anticongestive, anticonvulsive, antidiabetic, antidiarrhoeal, antiestrogenic, antifertility, antimicrobial, antifungal, antibacterial, anti stress, chemo preventive, haemaggultinating, hepatoprotective, radical scavenging, thyroid inhibitory, anti per oxidative, 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.\cite{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.\cite{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.\cite{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.\cite{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.\cite{3} The leaves are used in the preparation of ‘pattal’ (plates) considered to be pure and which are used particularly in religious ceremonies.\cite{9} They are also used for making cubs, bowls and beedi wrappers.\cite{8} The leaves are also used as appetizer, expectorant, astringent, anti-inflammatory, anodyne, aphrodisiac, in pimples boils, flatulence, colic worm infections, inflammations, arthralgia, haemorroidoids and night blindness.\cite{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.\cite{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.\cite{14} Flowers yields a brilliant yellow colouring matter due to presence chalcones.\cite{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
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. Seeds have anthelmintic property especially for roundworms and tapeworms. It is reported that the ethanolic extract of seeds of *B. monosperma*, on oral administration showed antifertility activity in mice and rats. The whole plant is used for timber, resin, fodder, medicine, and dye. 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.

Chemical screening of the parts of the species has shown the presence of flavonoids, chalcones, linoleic acid and unsaturated fatty acids. Plant contains cantharic acid, proanthocyanidins, L-β -Phenylalanine derivatives, aleuritic acid, palasitrin, and major glycosides as Butrin, alanind, allophanic acid, butolic acid, cyanidin, histidine, lupenone, lupeol, (−)-medicarpin, miroestrol, palasimide and shellolic acid. Roots contain glucose, glycine, a glycoside and an aromatic hydroxy compound. The euphane triterpenoid 3a-hydroxyeuph-25-ene and the alcohol 2, 14 dihydroxy-11, 12-dimethyl-8-oxo-octadec-11 enylcyclohexane, 3-Z-hydroxyeuph-25-ene, Stigmasterol-e-D-glucopyranoside and nonacosanoic acid has been reported in the stem. Stem bark containing β-sitosterol, kntannic acid, gallic acid, pyrocatechin and stigmasterol. 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). The leaves contains Glucoside, 3, 9-dimethoxypterocapran reported in ethyl acetate fraction of methanol extractives of leaves and hexane fraction of methanol extractives yielded 3-alpha hydroxyeuph-25-enylheptacosanoate. Flavonoids, chalcones, tannins. The phytoconstituents reported in the flower are triterpene, butein, butin, isobutrin, coreopsin, isocoreopsin (butin 7-glucoside), sulphurein, isomonomospermoside, chalcones, auronos, flavonoids (palasitrin, prunetin) chalkiness, aureoles, flavonoids (palasitrin, prunetin) and steroids. The compounds reported in seed are plasmatic, linoleic acid, α-anryrin, β-sitosterol its glucoside and sucrose, 8-lactone of n-heneicosanoic acid, anti helmintic principle compound palasonin and its L-β-phenylalanine derivative. Oil (yellow, tasteless), proteolytic, lypoletic enzymes, plant proteinase and polypeptidase (similar to yeast tripsin), a nitrogenous acidic
compound and monospermoside (butein 3-e-D-glucoside).[16] From seed coat allophonic 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]

Botanical classification[15]

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae – Plants</th>
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<tr>
<td>Sub-kingdom</td>
<td>Tracheobionta – Vascular plants</td>
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<tr>
<td>Super-division</td>
<td>Spermatophyta – Seed plants</td>
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<td>Division</td>
<td>Magnoliophyta – Flowering plants</td>
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<td>Magnoliopsida – Dicotyledons</td>
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<td>Fabales</td>
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<td>Family</td>
<td>Fabaceae – Pea family</td>
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<td>Genus</td>
<td>Butea Roxb.ex Wild. – Butea</td>
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<td>Species</td>
<td>monosperma (Lam.)</td>
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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± 2°C and relative humidity 50 ± 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° ± 3°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 cm × 10 cm TLC plates pre-coated with 0.25 µm 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 cm × 10 cm) previously saturated with vapours of mobile phase for 20 min. The plates were dried in air and visualized under λ 254 nm and λ 366 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 WinCATs software as shown in Figure 7-12.

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

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

366 nm

After derivatization with Anisaldehyde sulphuric acid reagent

Figure 10

Figure 11

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

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

<table>
<thead>
<tr>
<th>Wave-length</th>
<th>Ethyl acetate extract</th>
<th>Ethanol extract</th>
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<tbody>
<tr>
<td></td>
<td>Stem bark</td>
<td>Small branches</td>
</tr>
<tr>
<td>254</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.13, 0.15, 0.35, 0.51</td>
<td>0.07, 0.12, 0.15, 0.21</td>
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<tr>
<td></td>
<td>0.60</td>
<td>0.28, 0.31, 0.35, 0.38</td>
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<tr>
<td></td>
<td></td>
<td>0.47, 0.66, 0.70, 0.76</td>
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<tr>
<td></td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td>366</td>
<td>0.09, 0.20, 0.34, 0.40, 0.49, 0.72</td>
<td>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</td>
</tr>
<tr>
<td>Visible light after derivatization</td>
<td>0.08, 0.12, 0.19, 0.21, 0.36, 0.41, 0.48, 0.59, 0.62, 0.91, 0.95</td>
<td>0.12, 0.19, 0.36, 0.48, 0.59, 0.62, 0.91</td>
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</tbody>
</table>

**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 $R_y$ 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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