ABSTRACT

*Bombax ceiba* is commonly known as silk cotton tree and semal which belongs to family Bombacaceae. *B. ceiba* is an important medicinal plant of tropical and subtropical India. The plant name *Bombax ceiba* is derived from Greek word “Bombax” meaning silkworm and the word “ceiba” is the South American vernacular name for silk cotton tree. Its medicinal usage has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani. It has wide range of medicinal and pharmacological application. Our results revealed that the chromatographic fingerprint combined with similarity measurement could efficiently identify and distinguish *B. ceiba* stem bark and small branches. The phytochemical fingerprint profiling of stem bark and small branches of *B. ceiba* were found similar as an official part of *B. ceiba* 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. ceiba* species and adulterants.

**KEYWORDS:** *Bombax ceiba* L., HPTLC–UV detection, phytochemical fingerprint profiling analysis.

**Abbreviations:** HPTLC–UV, high performance thin layer chromatography-ultra violet detection; *R*<sub>f</sub>, retention factor; min., minutes; St. Bk., stem bark., Sm. Br., small branches;
INTRODUCTION

Bombax ceiba Linn (Fig.1) is a deciduous tree belongs to family Bombacaceae commonly known as silk cotton tree and semal. The plant name Bombax ceiba is derived from Greek word “Bombax” meaning silkworm and the word “ceiba” is the South American vernacular name for silk cotton tree.[1] This tree has its importance since ancient time. In 'Mahabharata' it is related to ‘Pitamaha’ according to which after erection the world was situated or kept under the tree ‘Salmali’ and in the 'Yajnavalkya' it is mentioned as one of the trees of the infernal regions. It is a large deciduous tree with tall trunk and spreading crown. The tree reaches up to 40 meter in height and 2 meter in diameter with the clear bole of 24-30 meter and girth up to 6 meter or more. Large trees are invariably buttressed at the base. Trunk and branches of young trees are covered with large woody conical, stout and hard prickles. Stem buttresses at the base and go up to 5-6 meter in height. Bark is pale ashy to silver grey or brown in color and 1.8-2.5 cm thick. The leaves are large, palmate, spreading, glabrous, digitate with a common petiole. Leaves are 15-30 cm long in size with 3-7 leaflets. The leaflets are lanceolate, acuminate, more or less coriaceous and entirely glabrous. Flower are numerous, large, fleshy, bright crimson, yellow or orange containing many seeds with long, dense silky hairs appearing in the months of January to March. Flower has the thick, fleshy and cup shaped sepals. It bears five petals in one flower which are 7.5-15 cm long. Seeds are smooth, black or gray embedded in long white wool. The pods are oval in shape and about 10-18 cm in length. The plant produces the gum which is light brown in color resembling the galls, and gradually becomes opaque and dark brown in color. The various part of B. ceiba such as roots, leaves, seed, stem bark, flower, fruit and gum are documented to possess medicinal properties in ethnobotanical surveys conducted by ethno botanist and in traditional system of medicine such as Ayurveda.[2] It is one of the important medicinal plants in tropical and subtropical India and also occurs in Sri Lanka, Pakistan, Bangladesh, Myanmar, Malaysia, Java, Sumatra and Northern Australia. It is widely found in temperate Asia, tropical Asia, Africa and Australia. In India, it can be found at altitudes upto1500 m. In peninsular India, the tree is very common in the dry as well as moist deciduous forests and near rivers. The tree is a strong light demander and fast growing. It grows best on deep sandy loams or other well-drained soils, particularly in valleys, in regions receiving 50 to 460 cm annual rainfall well distributed throughout the year. The tree prefers deep, sandy loam derived from granite but shows maximum development in deep, alluvial soil of the valleys. It grows on a well-drained hill slopes.[3-5] Natural regeneration takes place through seeds. B. ceiba showed the presence of compounds such as carbohydrates, proteins, calcium oxalate, volatile oils, starch, saponin,
phenols, saponins, xanthoproteins, triterpenoids, tannins and flavonoids.\cite{6} Chemical investigation on the root bark of *B. ceiba* shows presence of sitosterol β-D-glucoside, β-sitosterol, lupeol, 7-hydroxycadaleine, 1-methyl ether and 1, 2-dimethylether of isohemigossypol, 8-formyl-7-hydroxy-5-isopropyl-2-methoxy-3-methyl-l, 4-naphthaquinon, and the lactone of 8-carboxy-7-methyl-6-hydroxy-5-isopropyl-3-methyl-1-naphthol. The phytochemical studies on the roots of *B. ceiba* revealed the presence of lupeol, n-triacontanol, 7-hydroxy cadaleve triacontanol, β-sitosterol, 3',4',5,7-tetrahydroxy-6-methoxyflavan-3-O-β-D glucopyranosyl-D-xylopyranose, 1,6-dihydroxy-3-methyl-5-isopropyl-7-methyl-8-naphthalene carboxylic acid (8→1) lactone, 5,7,3,4 tetrahydroxy-6-methoxy flavon-3-O-β-D glucopyranosyl-α-D-xylotyranoside sesquiterpenoids. From the stem bark Shamimcin, 1‘’’’, 1’’’’’’-bis-2-(3, 4- dihydroxyphenyl)-3, 4-dihydro-3, 7-dihydroxy-5-O-xylopyranosyloxy-2H-1-benzopyran along with lupeol and β-sitosterol were isolated. Isolation and characterization resulted in the identification of two compounds from the extracts of stem barks of *B. ceiba* these compounds were lup-20 (29) en-3b-ol and 2-hexyl-7, 8-dimethyl-1, 4-naphthaquinone.\cite{7} Flowers of *B. ceiba* showed the presence of β-sitosterol, β-D-glucoside of β-sitosterol, henriciacontane, henriciacontanol, traces of essential oil, kaempferol, quercetin, polysaccharide-D-galactose, L-arabinose, L-rhamnose, pelargonidin-5-β-D-glucopyranoside,cyanidin-7-methylether-3-β-D-glucopyranoside, three biosides, viz., 24-β-ethylcholest-5-en-3-β-o-a-Larabinosyl-(1→6)-β-D-glucopyranoside, 3,5-dihydroxy-4'-methoxyflavone-7-o-a-L-rhamnopyranosyl-(1→6)-P-D-glucopyranoside, 4',5,7-D-trihydroxyflavone-3-0-β-D-glucopyranosyl-(1→4)-a-L-rhamnopyranoside and 8-formyl-6, 7-dihydroxy-5-isopropyl-3-methyl-1-naphthol.\cite{8,9} Fresh leaf contains shamimin (C-flavonol glycoside) and dried leaf extracts of the plant were subjected to chemical investigation, which led to the isolation of three new compounds 4-C-β-D Glucopyronosyl-1,3,6,8-tetrahydroxy-7-O-(4”'-hydroxybenzoyl)-9H-xanthen-9-one, 2-C-β-D Glucopyronosyl-1, 6, 7-trihydroxy-3-O-(4”'-hydroxybenzoyl)-9H-xanthen-9-one, 4-C- β-D-Glucopyronosyl-1, 6, 8-trihydroxy-3, 7-di-O-(4”'-hydroxybenzoyl)-9H-xanthen-9-one and one known compound mangiferin.\cite{10,12} The seed contain n-hexacosanol, palmitic acid, octylpalmitate, gallic acid, tannic acid, 1-galloyl-β-glucose, ethyl gallate, a mixture of α, β-and γ- tocopherol, carotenoids and lupeol. The oil from the seed was found to contain 94.5 percent mixed fatty acid composed oleic acid as a major constituent, along with myristic, palmitic, arachidic and linoleic acid.\cite{10,11,13} The phytochemical studies on the Gum of *B. ceiba* revealed the presence of gallic and tannic acids, L-arabinose, D-galctose, D-galacturonic acid and 6-0-(P-D-galactopyranosyluronic acid)-D-galactopyranose, 2,3,4,6-tetra- 2,6-di and 2,4-di-o-methyl-o-D-galactase and 2,3,5-tri
and 2,5-di-o-methyl-L-arabinose and aldobiuronic acid.[14] From the heart wood, 7-Hydroxy-5-isoproyl-2-methoxy-3-methyl-1, 4-naphthaquinone was isolated.[13, 15]

Almost all the parts of *B. ceiba* are of medicinal importance and used traditionally for the treatment of various ailments. *B. ceiba* having different pharmacological activities like anti-inflammatory, anti-diabetic, anti-obesity, hypotensive, antioxidant, antiangiogenic, antimicrobial, cytotoxicity, aphrodisiac and antipyretic. The plant is used in dysentery, menorrhagia, skin troubles, haemorrhoids, for the treatment of snake bite, scorpion sting, boils, leucorrhoea, internal bleeding, calculus affections, chronic inflammation, ulceration of bladder and kidney, gonorrhrea, haemoptysis, influenza, enteritis, pulmonary tuberculosis, cystitis and catarhal affections bleeding piles. Different parts of *B. ceiba* have been used in traditional Indian medicine. In Ayurvedic system of medicine plant of *B. Ceiba* is most admired and considered as good remedy for tvakdosa (skin disease), Asmari (urolithiasis), Raktarsa (bleeding piles), Daha (burning sensation), Vranasotha (wound), Atisara (Diarrhea), Arsa (autosomal recessive), Dantavikara (crooked teeth), Mukhapaka-vrana (Inflammation of the mouth).[12]

Root of *B. ceiba* is used in treatment of sexual weakness including seminal disorder diarrhoea, impotency, abdominal pain, gonorrhrea and also taken in debility and consumption.[11, 16] Young roots are especially used in diarrhoea, dysentery, urinary troubles, gynaecological problems, bladder disorders, heart diseases, diabetes and impotence.[17] Root bark is used as a tonic in case of sexual debility and also as nerve rejuvenator.[16] Stem bark is employed against inflammation, acrid, expectorant, diuretic demulcent, mucilaginous, emetic, slightly astringent and tonic. It is used superficially on face in facial complaints such as freckles, acne vulgaris, for skin eruptions and other cutaneous as well as pigmentational disorder and also on swelling, boil and burning sensation, to treat ulcer, healing wounds and muscular injury, also shows antiangiogenic activity. Demulcent, tonic and expectorant, paste of bark shows anti inflammatory activities. It is used to treat asthma, cattle wounds, pimples, urinary disorder, excessive vaginal bleeding and intestine bleeding.[17-19] Leaves are utilized for strangury, skin eruption, weakness, diarrhea and its paste is applied at the bitten spot in case of snake bite.[11, 20] Flowers are applied for skin troubles, as an astringent, splenomegaly, haemorrhoids and in snake bite.[11] Dry young fruit are beneficial in calculous affection, chronic inflammation, ulceration of the bladder and kidneys including strangury and other forms of dysuria.[11] Seeds are taken for gonorrhoea, paste prepared in water was applied on
small-pox boils, treatment of serious skin diseases like Leprosy. Seed oil used in the manufacture of soaps and lubrication substances. Gum resin is used in uterine disorder in traditional medicine, acute dysentery, stimulant, tonic, demulcent, anti dysenteric, analgesic and useful in giardiasis and diarrhoea. Influenza, blood vomiting and menorrhagia.

Taxonomic / Scientific Classification

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MATERIALS AND METHODS

Plant Materials and Chemicals
Plant materials i.e. stem barks (Fig. 2) and small branches of stem (Fig. 3) of *B. ceiba* 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. ceiba* were performed at room temperature (28°C ± 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. ceiba* (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 vapors 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 (V 1.4.2; CAMAG). HPTLC of ethyl acetate extract and alcoholic extract of both drugs were performed with same procedure in the mobile phase of *Toluene: Ethyl acetate 8: 2 (v/v)* and
Toluene: Ethyl acetate 6:4(v/v) 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. ceiba* (1= St. Bk.; 2= Sm. Br.)

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

No such type of study was found in literature for comparative phytochemical study of stem bark versus small branches of *B. ceiba* Linn by using High Performance Thin Layer Chromatographic-Ultra Violet detection Method. Comparative study of TLC fingerprints of stem bark and small branches of *B. ceiba* 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 under 254 nm showed no band in stem bark and one band in small branches, thus no band was found
similar. Under 366 nm UV detection, stem bark and small branches showed four and five band respectively, out of which, three bands at $R_f 0.36$ (red), 0.40 (red) and 0.64 (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 three and five bands respectively, out of which, three bands at $R_f 0.15$ (violet), 0.28 (blue), 0.42 (violet) were found similar as represented in Table 1 and Fig. 4-6.

Ethyl acetate extract of stem bark and small branches (stem) under 254 nm, both extract showed one band at different $R_f 0.81$ and 0.74. Therefore, no similar bands were observed. Under 366 nm UV detection, stem bark and small branches showed eleven and twelve bands respectively, out of which, nine bands at $R_f 0.18$ (blue), 0.34 (blue), 0.47 (red), 0.63 (red), 0.67(blue), 0.71 (red), 0.75 (red), 0.81(red) and 0.91(red) were found similar. After derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, stem bark and small branches showed five and four bands respectively, out of which four bands at $R_f 0.38$ (blue), 0.58 (blue), 0.70 (blue) and 0.93 (blue) were found similar as showed in Table 1 and Fig. 7-9.

Phytochemical fingerprints of ethanol extract of stem bark and small branches under UV detection at 254 nm, showed no similar band. While under 366 nm UV detection, stem bark and small branches showed seven and eight bands respectively, out of which, three bands at $R_f 0.71$ (blue), 0.80 (red) and 0.86 (red) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, stem bark and small branches showed six and five bands respectively, out of which four bands at $R_f 0.47$, 0.57, 0.68 and 0.93 (all are blue) were similar in both parts (St. Bk. and Sm. Br.) as evident in Table 1 and Fig.10-12.

**CONCLUSION**

The phytochemical fingerprint profiling of stem bark and small branches of *B. ceiba* were found similar as an official part of *B. ceiba* 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. TLC phytochemical fingerprint profiling of *n*-hexane, ethyl acetate, ethanolic extracts of stem bark and small branches of *B. ceiba* 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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REFERENCES


