

DEVELOPMENT OF HPTLC-UV METHOD FOR COMPARATIVE PHYTOCHEMICAL STUDY OF STEM BARK *VERSUS* SMALL BRANCHES OF *STREBLUS ASPER* LOUR

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ABSTRACT

Streblus asper Lour belongs to the family Moraceae. It is a small tree found in tropical countries, such as India, Sri Lanka, Malaysia, the Philippines and Thailand. *S. asper* is a medicinal plant from Thailand used in folk medicine for the treatment of several inflammatory diseases, treat wounds, skin diseases, filariasis, leprosy, toothache, fever, diarrhea, dysentery. The chromatographic fingerprints of stem bark and small branches of *F. racemosa* were developed and validated for comparison of phytochemicals. Our results revealed that the chromatographic fingerprints combined with similarity measurement could efficiently identify and distinguish *S. asper* stem bark and small branches. The phytochemical fingerprint profiling of stem bark and small branches of *S. asper* were found similar as an official part of *S.*

asper 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 *S. asper* species and adulterants.

KEYWORDS: *Streblus asper*, 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

Streblus asper is a medicinal plant from Thailand used in folk medicine for the treatment of several inflammatory diseases. *S. asper* Lour belongs to the family Moraceae.^[1] *S. asper* is a tree known by several common names, including Siamese rough bush, khoi, serut, and toothbrush tree. It is a medium-sized tree native to dry regions in Thailand, India, Malaysia, and Vietnam. In the Republic of the Philippines, it is commonly known as "bogta-e" or "bogtalay".^[2,3] A bushy, small, evergreen tree with milky juice. Leaves alternate, elliptic or obovate, 5-10 cm long, irregularly crenate, acute or shortly acuminate, very rough on both surfaces, petiole very short. Flowers dioecious, axillary; male flowers in globose heads, 7.5 mm diam., female flowers solitary, inconspicuous, long peduncled. Fruit 1-seeded berry, 5 mm diam., yellow when ripe.^[2-6]

S. asper is a rich source of cardiac glycosides. More than 20 cardiac glycosides have been isolated by Reichstein and co-workers from the root bark of *S. asper* according to literature,^[7] and 15 such compounds were structurally characterized, mainly as a result of the application of degradative techniques, namely kamloside, asperoside, strebloside, indroside, cannodimemoside, strophalloside, strophanolloside, 16-O-acetylglucogitomethoside, glucogitodimethoside, glucokamloside, sarmethoside and glucostrebloside. The other glycosides reported from the roots include b-sitosterol-3-O-b-d-arabinofuranosyl-O-a-l-rhamnopyranosyl-O-b-d-glucopyranoside, lupanol-3-O-b-d-glucopyranosyl-^[1-5]-O-b-d-xylofuranoside and vijaloside, i.e. periplogenin-3-O-b-d-glucopyranosyl-^[1-5]-O-b-d-xylopyranoside.^[8-11] From the stem bark of this plant, a-amyrin acetate, lupeol acetate, b-sitosterol, a-amyrin, lupeol and diol, strebloside and mansonin have been isolated. A pregnane glycoside named sioraside has also been isolated. n-Triacontane, tetraiacontan-3-one, b-sitosterol, stigmasterol, betulin and oleanolic acid were identified from the aerial parts. An unidentified cardenolide, b-sitosterol, a-amyrin and lupeol were isolated from root bark and leaves.^[12,13] The volatile oil from fresh leaves of *S. asper* was obtained in 0.005% yield as a brown liquid. The major constituents of the volatile oil were phytol (45.1%), a-farnesene (6.4%), trans-farnesyl acetate (5.8%), caryophyllene (4.9%) and trans-trans-a-farnesene (2.0%). The other constituents were a-copaene, b-elemene, caryophyllene, geranyl acetone, germacrene, d-cadinene, caryophyllene oxide and 8-heptadecene.^[14]

The tree has a number of uses. It has been important in papermaking in Thailand for seven hundred years. Virtually all of the ancient Thai documents still in existence are written on the

bark of this tree. The paper is durable even in the local high-humidity climate. It does not burn easily and it is resistant to yellowing and insect damage ^[15] In Vietnam traditional woodworking uses the coarse texture of the leaves as natural sandpaper. The fruits are sweet and edible, leaves used as tea and also use for scrubbing utensils, seeds are used in traditional medicine, as fodder for animal, bonsai and topiary material, firewood and charcoal. The twigs are chewed to make brushes and are said to cure pyorrhea.^[16] Various parts of the plant are used in Ayurveda and other folk medicines for the treatment of different ailments such as filariasis,^[17] leprosy, toothache, diarrhoea, and cancer. It is a well known and documented ethnomedicinal plant. It has been used in the past as an oral hygiene product and for this reason it is also known as the toothbrush tree. A twig or stick about eight inches long with a frayed or mashed end to increase the cleaning surface was used as a tooth cleaning aid up until the middle of the twentieth century when the cheap and more practical plastic brush with a toothpaste become common throughout the world. It is the main active ingredient of a popular brand of a herbal, dark brown toothpaste in Thailand. Root of this plant is used to cure unhealthy ulcers and sinuses and as antidote to snake bite, in epilepsy and obesity^[18] Stem is very effective in toothache and stem bark is used in fever, dysentery and diarrhea, stomachache and urinary complaints, useful in piles, edema and wounds, decoction effective against lymphadema, chylurea and other effects of filariasis. Leaves and fruit of this plant are useful in various eye complaints. Leaves are used in urinary inflammation and as a galactagogue. The crushed leaves administered as diuretic and in the treatment of leucorrhoea. Leaf juice is used by the Garo of Madhupur against dysuria and dysentery^[19] Milky juice/latex of the plant possesses antiseptic and astringent properties and can applied to chapped hands and sore feet it is also used in pneumonia, swells of cheek and reported to act as a sedative in the treatment of neuralgia. Seeds are applied in epistaxis and diarrhea. Whole plant is found effective in Cancer, cholera, colic, diarrhea, dysentery and menorrhagia, epilepsy and inflammatory swellings.^[20]

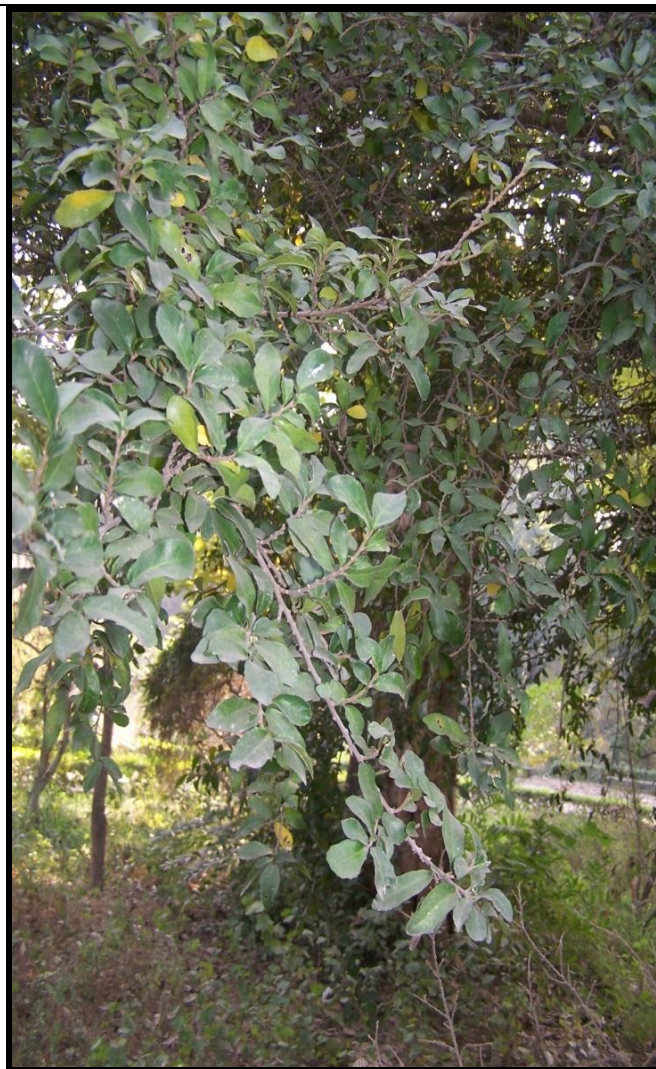


Figure 1:



Figure 2: Stem Bark.



Figure 3: Small Branches.

Taxonomic / Scientific Classification

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Order	Rosales
Family	Moracea
Tribe	Moreae
Genus	Streblus
Species	<i>Streblus asper</i>

MATERIALS AND METHODS

Plant Materials and Chemicals

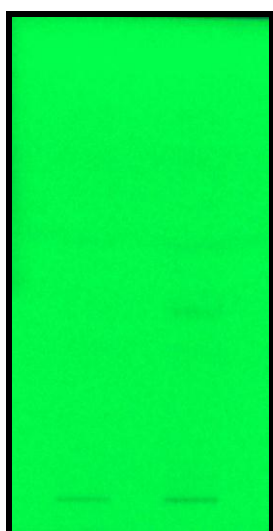
Plant materials i.e stem barks (Fig.2) and small branches of stem (Fig.3) of *S. asper* were collected in December 2013 and authenticated by Dr. R. K. Tiwari, Research Officer, Pharmacognosy, National Veterinary Research Institute & Hospital, Lucknow. All chemicals (AR grade) and TLC plates were purchased 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 *S. asper* 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 *S. asper* (10 g each) were extracted three times ($3 \times 50\text{ mL}$) 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.

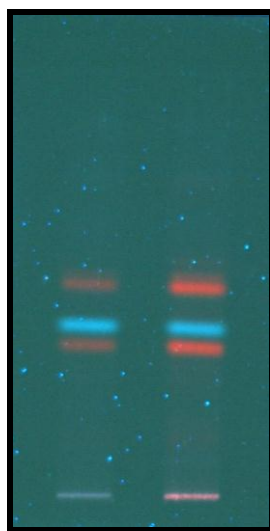
HPTLC-UV detection Method

High Performance Thin Layer Chromatography was performed on $10\text{ cm} \times 10\text{ cm}$ TLC plates pre-coated with $0.25\text{ }\mu\text{m}$ 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 vapors of mobile phase for 20 min. The plates were dried in air and visualized under $\lambda\text{ }254\text{ nm}$ and $\lambda\text{ }366\text{ nm}$ 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 (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 $\lambda\text{ }254\text{ nm}$, $\lambda\text{ }366\text{ nm}$ and white light using CAMAG Reprostar and WinCATs software as shown in Figure 7-12.



1 2

254 nm

Figure 4

1 2

366 nm

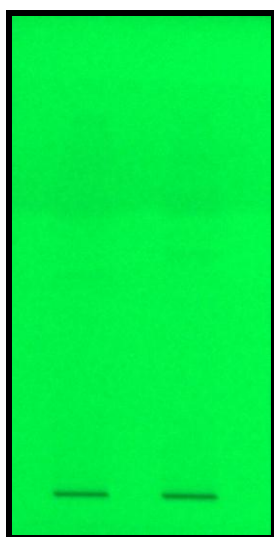
Figure

1 2

After derivatization

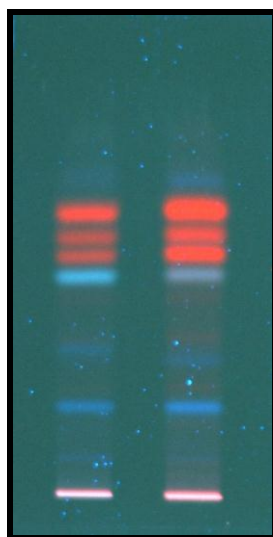
Figure 6

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



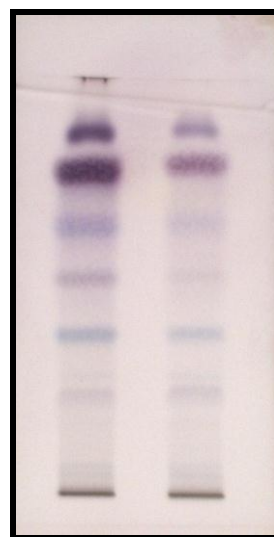
1 2

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 *S. asper* (1= St.; 2= Sm. Br.)

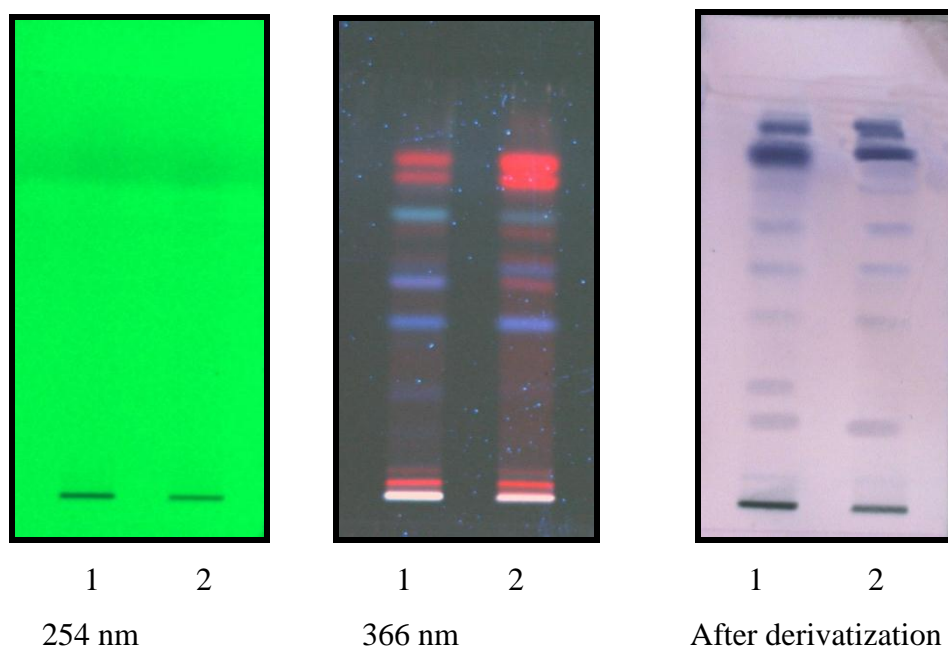


Figure 10

Figure 11

Figures 12

Figure 10-12: TLC fingerprint of ethanol extract of *S. asper* (1= St.; 2= Sm. Br.)Table 1: R_f value of phytochemicals present in *n*-hexane, ethyl acetate and ethanol extract of *S. asper* (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	No band	No band	No band	No band	No band	No band
366	0.36,0.40,0.51	0.36,0.40,0.49,0.51	0.10,0.22,0.34,0.51,0.58,0.62,0.66,0.74	0.10,0.22,0.32,0.38,0.50,0.58,0.62,0.66,0.74	0.09,0.26,0.42,0.51,0.64,0.67,0.78,0.81	0.09,0.42,0.51,0.55,0.64,0.67,0.78,0.84
Visible light after derivatization	0.16,0.28,0.39,0.48,0.52,0.72,0.88	0.16,0.28,0.39,0.48,0.52,0.72,0.88	0.07,0.26,0.40,0.52,0.64,0.80,0.88	0.07,0.26,0.40,0.52,0.64,0.80,0.88	0.07,0.21,0.27,0.44,0.56,0.64,0.82,0.89	0.07,0.20,0.44,0.56,0.82,0.89

RESULTS AND DISCUSSION

No such study was found in literature for comparative phytochemical study of stem bark versus small branches of *S. asper* by using High Performance Thin Layer Chromatographic-Ultra Violet detection Method. Comparative study of TLC fingerprints of stem bark and small branches of *S. asper* 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 small branches, thus no band was found similar. Under 366 nm UV detection, stem bark and small branches showed three and four band respectively, out of which, three bands at R_f 0.36 (red), 0.40 (blue) and 0.51 (red) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, total seven bands were visible in both the parts and all seven bands at R_f 0.16 (blue), 0.28 (blue), 0.39 (blue), 0.48 (blue), 0.52 (blue), 0.72 (violet), 0.88 (violet) were similar.

Ethyl acetate extract of stem bark and small branches (stem) under 254 nm showed no band in stem bark and small branches, thus no band was found similar. Under 366 nm UV detection, stem bark and small branches showed eight and nine bands respectively, out of which, six bands at R_f 0.10 (blue), 0.22 (blue), 0.58 (red), 0.62 (red), 0.66 (red), 0.74 (blue) were found similar. After derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, total seven bands were visible in both the parts and all seven bands at R_f 0.07 (blue), 0.26 (blue), 0.40 (blue), 0.52 (violet), 0.64 (blue), 0.80 (violet), 0.88 (violet) were similar.

Phytochemical fingerprints of ethanol extract of stem bark and small branches under UV detection at 254 nm showed no band in stem bark and small branches, thus no band was found similar. While under 366 nm UV detection, total eight bands were visible in both the parts, out of which, six bands at R_f 0.09 (blue), 0.42 (red), 0.51 (red), 0.64 (blue), 0.67 (red), 0.78 (red) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, stem bark and small branches showed eight and six bands respectively, out of which five bands at R_f 0.07, 0.44, 0.56, 0.82, 0.89 (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 *S. asper* were found similar as an official part of *S. asper* 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 *S. asper* have been given an idea about the presence of various photochemical in their reported parts. The TLC spots

provided valuable clue regarding presence or absence of various photochemical or metabolites of the plants.

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