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Pharmacognostical Standardization, Phytochemical Characterization and HRLC– MS-Based Metabolite Profiling with Antimicrobial Evaluation of Streblus as per Lour

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ABSTRACT: Streblus Asper, also known as toothbrush tree or fig mulberry, belonging to the family Moraceae serves as a versatile resource due to its abundance of bioactive compounds, it mainly belongs from the central or northeastern India. For long Streblus asper has been utilised as traditional form of medicine for the treatment of infections, inflammations, wounds, oral diseases and various other disorders. In this paper, Streblus asper was subjected to extraction through the process of Soxhlet extraction, the yield of extraction was found out to be 8%. then the extract was lyophilised. Various Physiochemical Standardization were performed such as phytochemical analysis, ash value, extractive value and loss of drying followed by Pharmacognostical studies such as Macroscopic characteristic and organoleptic Characteristic. The chemical profiling was performed using High Resolution Liquid Chromatography- Mass Spectroscopy (HRLC-MS) and the detected metabolites span a range of chemical classes including amino acids and their derivatives, polyamines, organic acids, lipids and fatty acid derivatives, and other bioactive compounds, while antimicrobial activity was evaluated using the disc diffusion method against Exigobacterium and Escherichia coli (E.Coli), The extract showed 1.6 mm zone of inhibition for Exigobacterium and 0.6 mm resistance for Escherichia Coli. This result supports that the Streblus asper plant possesses promising bioactive compounds that may contribute to future development.

1. INTRODUCTION

Humans rely on plants as their primary sources of sustenance and medicine. As of now, it has been reported that over 7000 plant species are palatable, either because they are found in the wild or because they can be manually cultivated [1]. Numerous countries advocate for the inclusion of medicinal plants in the diet as a daily or functional food, thereby enhancing overall health [3]. The presence of a variety of phytochemicals in plants enables them to provide a wide range of health benefits [2]. A variety of phytochemicals and micro and macronutrients in plants can serve as a source of vitality and wellness in terms of health benefits. The traditional use of plants is playing an important role acting as ethnomedicines amongst various tribes and communities [3]. Various plants and their by-products have become a major area of investigation for bioactive compounds with health benefits [3,4]. In this regard, many plant species known to have traditional health benefits are analysed and identified to determine the different pharmacological potential of the plants and also to identify the phytochemicals responsible for it.

Streblus asper Lour, is one such plant belonging to the family Moraceae. This plant is commonly known as “sheora” in Bengali [5,6], and “khoi” in Thai [7] and in Assamese this plant is called as ‘sora’[8]. *Streblus asper* is known for its medicinal properties; it is known as “Shakhotaka” in Ayurveda and “Piraayan” in Siddha. The plant is grown widely in Asia, majorly in India, Thailand, Sri Lanka, Philippines, China, and Malaysia, in open, wet, and dry fields, along with coastal regions [9,10]. It is used traditionally in leprosy, piles, diarrhea, dysentery, elephantiasis [11] and cancer [12]. *Streblus asper* serves as a versatile resource, with sweet edible fruits, leaves for tea and cleaning, medicinal seeds, animal fodder, ornamental potential, valuable timber, and as a source of fuel in the form of firewood and charcoal, mainly in Vietnam [8].

The current paper entails systematic studies of *Streblus asper* Lour., which include all the necessary experiments conducted, as shown in the flowchart below. These include gathering and standardizing of the plant by means of assessing its morphological parameters, moisture content, extracts, ash content, and preliminary phytochemical screening. In addition, the plant is extracted after which lyophilization is done on the extract obtained. Compound profiling is conducted using HPLC-MS, besides assessing antimicrobial and antibacterial activities

Description of plant.

A rigid evergreen shrub or small tree, plant parts with milky juice. Bark soft, light grey, irregularly ribbed Leaves simple, alternate, rigid, elliptic, or obovate, irregularly toothed, rough on both sides. Flowers axillary, minute, dioecious. Male flowers globose, minute, perianth campanulate, yellowish green. Stamens 4, long, inflexed in bud. Female flowers are very small, solitary or 2-4 flowers together. Styles 2, filiform, connate at base. Fruit a yellow 1-seeded berry, sub globose. Flowering January to March. Fruiting April to May [11,14,15].

Table no. 1: Taxonomical Classification of *Streblus asper* [13]

Kingdom	Plantae
Subkingdom	Tracheophyta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Family	Moraceae
Genus	<i>Streblus</i>
Species	<i>asper</i>

Table no. 2: Ethnomedicinal uses of different parts of *S. asper* [21]:

Sl. No:	Parts	Function
1	Root	As an application to unhealthy ulcers and sinuses and as antidote to snake bite [15], in epilepsy and obesity [16]
2	Stem	Toothache [17]
3	Stem Bark	Given in fever, dysentery and diarrhea [15,16], stomachache and urinary complaints [17], useful in piles, edema and wounds [16], decoction effective against lymphedema, chyluria and other effects of filariasis [16,18,19]
4	Leaves	Eye complaints [17]
5	Milky juice/latex	Antiseptic, astringent, applied to chapped hands and sore feet [15], in pneumonia and swells of cheek [17]
6	Fruit	Eye complaints [17]
7	Seeds	Epistaxis and diarrhea [20]

2. MATERIALS AND METHODS

2.1 Identification and authentication

The plant material has been successfully identified and collected from the appropriate source. Proper procedures were followed during collection to ensure sample authenticity, quality, and suitability. Collection was done from Lakhimpur, Assam and authenticated from Guwahati University, Department of Botany.

2.2 Extraction process

About 180 gm stems of *Streblus asper* were thoroughly washed with water to remove the surface impurities, then it was shade dried until completely dry. After that, the stems were powdered using a cutter mill to obtain the crude plant material of about 157.43 gm.

The method of extraction that is used is Soxhlet method of extraction, in which 50 gm of *Streblus asper* was taken and a suitable solvent was taken, in this case methanol was used and it was kept for 72 hours. The methanolic extract was collected and put in rotary evaporator to remove the methanol from the extract and make it concentrated, then the concentrated extract was given to The National Institute of Pharmaceutical Education and Research, Guwahati for the process of lyophilization and after 48 hours the dry extract was obtained. The percentage yield came to be 8%.

2.3 Physiochemical Analysis

Loss of drying: 2 gm of the crude drug was weighed and heated at 110° C for 4 hours, after that the petri dish was cooled in desiccator and weighed again [8].

$$\frac{\text{Final Weight}}{\text{Initial Weight}} \times 100 \quad \text{Eq. 1}$$

Extractive Value: There are two types of extractive values that are performed, that are alcohol extractive value and water extractive value [8].

- i. Alcohol Extractive Value: About 2g of the coarsely powdered air-dried drug was macerated with 10ml of C₂H₅OH in a closed flask for 24hrs, frequently shaken during the first 6hrs and allowed to stand for 18hrs. Thereafter it was filtered rapidly and 5ml of the filtrate was evaporated to dryness in a tared flat-bottom shallow dish, dried at 105°c, and weighed. The percentage of w/w of alcohol-soluble extractive was calculated.

$$\frac{\text{Final weight} - \text{initial weight}}{\text{Sample Taken}} \times 100 \quad \text{Eq. 2}$$

- ii. Water Extractive Value: About 2g of the coarsely powdered air-dried drug was macerated with 10ml of CHCl₃-H₂O in a closed flask for 24hrs, frequently shaken during the first 6hrs and was allowed to stand for 18hrs. Thereafter it was filtered rapidly and 5ml of the filtrate was evaporated to dryness in a tared flat-bottom shallow dish, dried at 105°c, and weighed. The percentage of w/w of water-soluble extractive was calculated concerning the air-dried drug.

$$\frac{\text{Final Weight} - \text{Initial Weight}}{\text{Sample Taken}} \times 100 \quad \text{Eq. 3}$$

- iii. Ash Value: About 2g of crude powder was taken and weighed in a tared silica dish which was previously ignited and weighed. Scattered the powdered drug at the bottom of the dish and ignited it by gradually increasing the heat up to 550°C until it is white indicating the absence of carbon. The percentage of ash was calculated by incinerating completely the crude powdered drug and comparing its weight with the initial weight of the sample [8].

$$\frac{\text{Final weight} - \text{Initial weight}}{\text{Sample taken}} \times 100 \quad \text{Eq. 4}$$

2.4 Phytochemical screening

Phytochemical screening of the extract of *Streblus asper* was performed with slight modification to identify different secondary metabolites like alkaloids, carbohydrates, phlobatannins, glycosides, cardiac glycosides, oils and fats, proteins and amino acids, flavonoids, phenolic compounds, resins, tannins, saponins, gums and mucilages, triterpenoids, quinones, anthraquinones, carboxylic acids, coumarins, and anthocyanins. [8,24,25].

2.5 Pharmacognostical studies

Macroscopic Characters: The morphology of leaf part of the plant was studied thoroughly. Colour, characteristics of petiole, leaf base, leaf lamina, apical part of leaf, the margin, venation and phyllotaxy were studied [22,23].

Organoleptic Characters: Organoleptic properties were determined through sensory analysis of the plant material. Fresh and dried plant material of *Streblus asper* Lour. was studied in terms of colour, odour, taste, and texture. [23]

2.6 High-Resolution Liquid Chromatography-Mass Spectrometry

Samples were analyzed using a Waters Alliance 2695 HPLC system (Waters, Milford, MA, USA) with an Accu Core C18 column (150 × 4.6 mm, 2.6 µm) at 40 °C, combined with Waters 2998 PDA detector and ESI-MS detector controlled using MassLynx 4.1. Solvent B (acetonitrile) and solvent D (aq. phase: 5% acetonitrile/water, 5 mM acetic acid) were used as mobile phases. Flow rate was set to 1.0 mL/min with a total run time of 40 min and an injection volume of 10 µL. The gradient program was: 0–1 min, 5% B; 1–10 min, linear to 30% B; 10–14 min, linear to 60% B; 14–16 min, 60% B; 16–24 min, linear to 80% B. The composition was held at 80% B until 32 min, then back to the initial condition of 5% B by 35 min, and re-equilibrated until 40 min. The PDA detector covered the wavelength range 200–450 nm and had additional single wavelength channels at 220, 240, 280, 350 and 410 nm. The mass spectrometric analysis was performed in positive and negative electrospray ionization modes with full scan acquisition in the m/z range 100–750. The capillary voltage was 3.5 kV (positive mode) and 2.5 kV (negative mode) with a cone voltage of 30 V, extractor of 3 V, source temperature of 120 °C and desolvation temperature of 350 °C. Desolvation gas and cone gas were nitrogen at flow rates of 650 L/h and 50 L/h. Data was acquired using MassLynx with an automatic scan time of 0.5 seconds. Data processing included a smoothing window of 3 (two passes) and spike removal on. Sample acquisition was preceded by calibration using the last calibration file in the system.

2.7 Anti-Microbial and Anti-Bacterial Studies

Sample preparation: The provided sample was grinded in mortal pestle into powder form. The extracts were weighed and dissolved in DMSO as the solvent. A stock extract was taken as concentrated.

Procedure: Serial dilutions of each of extract were made up to 10⁻¹, 10⁻², 10⁻³. (Dilution factor 10). 100 µl extract of concentrate was mixed with 900 µl of DMSO in each tube and final volume of all tubes was made up to 1000 µl. A total of 3 extract was taken for disc diffusion with 4 quadrant method. Using the Mueller Hinton base agar (medium) was prepared by dissolving required amount in distilled water and sterilized in an autoclave for 15 mins at 121°C at 15 psi and then directly taken inside Laminar Air Flow to maintain the sterile environment. Sterilized media was poured into petri-plates and allowed to get solidified.

Petri plates were inoculated with standardized inoculum of Gram positive (*Exiguobacterium*) and Gram negative (*E.coli*) respectively. Taking Gentamicin as positive controls at the center (30 mcg) against the given bacterium respectively. The plates were incubated at 37°C and observed after 24 hrs to 48 hrs for examining the zone of inhibition, around the disk. The inhibition zones were measured with Himedia MIC scale [8].

3. RESULTS AND DISCUSSION

3.1 Physiochemical Analysis: The results of different physical standardisation tests are given in the table below.

Table no. 3: Physical standardisation tests of *S. asper*

Sl.no.	Test	Result
1	Loss of drying	8%
2	Alcohol Extractive Value	15%

3	Water Extractive Value	4%
4	Ash Value	14%

3.2 Phyto-chemical Analysis: The phytochemical analysis of the plant extract it has been found that *Streblus asper* is a rich source of Alkaloid, Carbohydrate, Reducing Sugar, Cardiac Glycoside, Triterpenoids, Coumarins and Anthocyanins.

Table no.4: Phytochemical analysis tests of *S. asper*

Sl.no.	Test	Result
1.	Alkaloids	+
2.	Carbohydrate	+
3.	Reducing sugar	+
4.	Glycosides	-
5.	Cardiac Glycosides	+
6.	Oils and Fats	-
7.	Proteins and Amino acids	-
8.	Flavonoids	-
9.	Phenolic Compounds	-
10.	Resins	-
11.	Tannins	-
12.	Saponins	-
13.	Phytosterols	-
14.	Triterpenoids	+
15.	Terpenoides	-
16.	Coumarins	+
17.	Anthocyanins	+

3.3 Pharmacognostical Studies: This are used to identify and standardize medicinal plants by evaluating their macroscopic, microscopic, and organoleptic characteristics. These parameters help ensure the authenticity, quality, and purity of crude drugs.

Table no.5: Macroscopic Morphology of *S. asper*

Part	Inference
Stem	Woody, grey-brown, rough, fibrous.
Leaf	Simple, Rough, elliptic like ovate, 4-10 cm, thick midrib, dark green with milky latex

Table no. 6: Organoleptic properties of *S. asper*

Colour	Greenish- brown (leaf), brownish-yellowish (stem)
Odor	mild grassy
Taste	slightly bitter and astringent
Texture	very coarse

3.4 High-Resolution Liquid Chromatography-Mass Spectrometry: The Raw peak data were processed and filtered to remove noise and redundant features, yielding 21 putatively identified metabolites. Compound identities were assigned by matching exact mass and retention time against the KEGG metabolite database. The detected metabolites span a range of chemical classes including amino acids and their derivatives (Tyramine, L-2-Aminoadipate, Homoarginine, L-Methionine), polyamines (Spermidine, N1-Acetylspermidine), organic acids (2-Oxobutanoate, D-Glucuronate), lipids and fatty acid derivatives (Ximenic acid, Arachidonyltrifluoromethane, Methyl acetyl ricinoleate), and other bioactive compounds.

MS2 ES⁻ : BPI → Negative ion mode

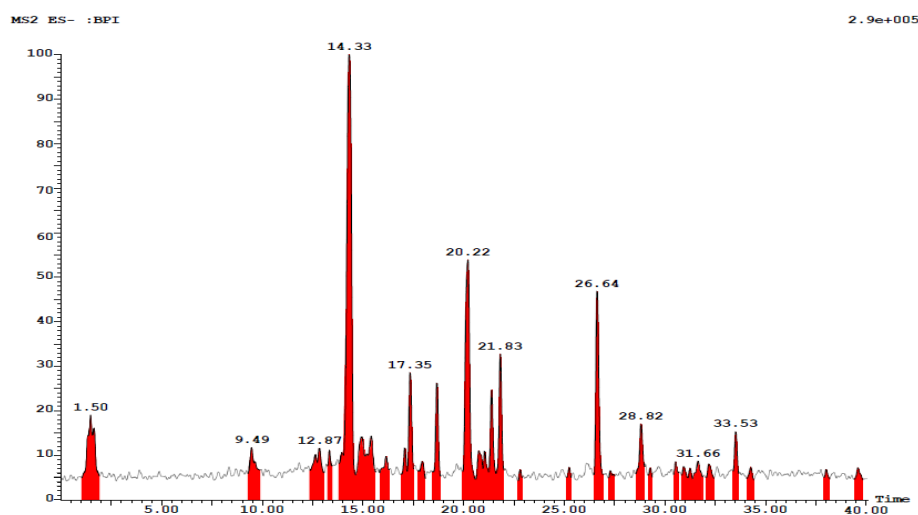


Fig 1 (a): Represents HR-LCMS Chromatogram in negative ion mode

MS2 ES⁺ : BPI → Positive ion mode

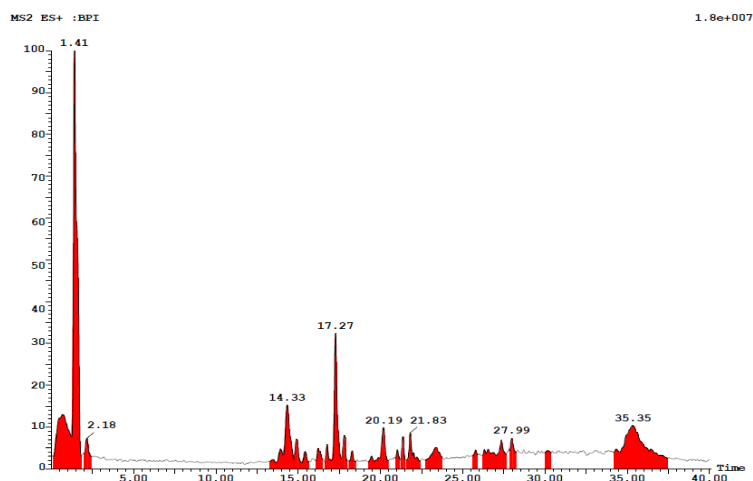


Fig 1 (b): Represents HR-LCMS Chromatogram in positive ion mode

Table no. 7: The m/z values, retention times (RT), and peak heights for each detected compound are summarised.

Sl.No.	Compound Name	Molecular Formula	m/z	RT (min)	Peak Height
1	2,3,4,5-Tetrahydridipicolinate	C ₇ H ₉ NO ₄	172.0849	1.351	61,002.63

2	Carumonam sodium	C ₁₂ H ₁₂ N ₆ O ₁₀ S ₂ ·2Na	511.0308	1.351	219,986.75
3	Tyramine	C ₈ H ₁₁ NO	138.1176	1.407	944,937.00
4	N1-Acetylspermidine	C ₉ H ₂₁ N ₃ O	188.1745	1.463	330,869.00
5	L-2-Aminoadipate	C ₆ H ₁₁ NO ₄	162.0410	1.477	88,238.19
6	Spermidine	C ₇ H ₁₉ N ₃	146.1463	1.505	857,291.00
7	2-Oxobutanoate	C ₄ H ₆ O ₃	103.0124	1.547	143,040.38
8	Immevip	C ₉ H ₁₅ N ₃	166.1691	1.603	188,778.25

9	Ximenic acid	C ₂₆ H ₅₀ O ₂	395.4181	1.631	72,797.50
10	Homoarginine	C ₇ H ₁₆ N ₄ O ₂	189.1172	1.645	93,984.31
11	Phosphocreatine	C ₄ H ₁₀ N ₃ O ₅ P	212.0652	1.673	169,736.88
12	D-Glucuronate	C ₆ H ₁₀ O ₇	195.0330	2.149	60,457.94
13	Carnitine	C ₇ H ₁₆ N ₃ O	163.1461	4.124	102,957.50
14	L-(+)-Anaferine	C ₁₃ H ₂₄ N ₂ O	225.2294	9.390	58,600.50
15	19-(4-Hydroxyphenyl) nonadecanoate	C ₂₅ H ₄₂ O ₃	391.3552	9.922	95,537.00
16	Dimethisterone	C ₂₃ H ₃₂ O ₂	341.2677	12.093	166,771.88
17	Arachidonyltrifluoromethane	C ₂₁ H ₃₁ F ₃ O	357.2268	13.017	115,795.13
18	Methyl acetyl ricinoleate	C ₂₁ H ₃₈ O ₄	355.3091	14.264	65,865.94
19	Cyclohexanol	C ₆ H ₁₂ O	101.0946	28.618	722,179.00
20	L-Methionine	C ₅ H ₁₁ N ₂ O ₂ S	150.0468	35.915	80,317.50
21	Paramethadione	C ₇ H ₁₁ N ₃ O ₃	158.1079	38.282	449,034.50

3.5 Anti-microbial and Anti-bacterial study: The antimicrobial study was performed using well diffusion method with two bacterial strains that are *E.coli* and *Exiguobacterium* the activity index was found out.

Table no. 8: Antimicrobial study

Sl.no.	Concentration	Plant Extract	Bacteria	Antibiotic	Resistance
1	10 ⁻³	2 mg/ml	Exiguobacterium	28 mm	1.6 cm
2	10 ⁻³	2 mg/ml	E. coli	28 mm	0.5 cm

The Disc diffusion zone of inhibition method was used for studying antibacterial activity of the provided test sample (*Streblus Asper*) along with control antibiotic sample, Gentamicin 30 mcg against Gram positive (*Exiguobacterium*) and Gram negative (*E.coli*). The test sample was observed for antibacterial activity by measuring the zone of inhibition against the bacterium mentioned. The test sample shows resistance $\leq 1.6\text{mm}$ towards *Exiguobacterium* and $\leq 0.5\text{mm}$ towards *E.coli*. The positive control, antibiotic Gentamicin 30 mcg showed clear zone of inhibition.

4. CONCLUSION

The present study demonstrates that *Streblus asper* Lour. is a pharmacologically significant plant possessing notable antioxidant and antimicrobial activities, as evidenced by the obtained results. These findings highlight its potential as a valuable source of bioactive compounds. Further detailed investigations are warranted to explore its full medicinal and therapeutic potential, which may facilitate the development of effective formulations for the treatment of various ailments and contribute to the advancement of healthcare.

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AUTHOR CONTRIBUTIONS

Sudarshana Borah: Conceptualization, Investigation, Supervision.

Ananya Saha: Methodology, Laboratory Analysis.

Rishika Choudhury: Methodology, Laboratory Analysis.

Joyjeet Dey: Writing-Review & Editing.

Pallab Kumar Nath: Data Curation, Writing-Review & Editing.

Sabnam Nargis: Data Curation, Writing-Review & Editing.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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