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Ethnopharmacological Validation Of Streblus Asper Lour. (Family: Moraceae) Used In The Traditional Therapeutic Of Assam

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Abstract:
Streblus asper is a well-known ethnomedicinal plant extensively used in traditional healthcare systems of India and Southeast Asia. The present study aimed to quantitatively evaluate key phytochemicals and assess the antioxidant and antibacterial potential of aqueous leaf and branch extracts of *S. asper*. Quantitative phytochemical analysis revealed the presence of high levels of phenols, flavonoids, tannins, and saponins in both extracts, with leaf extracts showing significantly higher concentrations of phenols (87.41 ± 0.839 µg/mg), flavonoids (84.64 ± 0.979 µg/mg), and tannins (83.91 ± 0.464 µg/mg) compared to branches. Antioxidant activity evaluated by DPPH radical scavenging assay showed notable activity in leaf (55.05 ± 0.467%) and branch (45.86 ± 0.664%) extracts, indicating strong free radical scavenging potential. Antibacterial activity assays demonstrated that both extracts were effective against *Bacillus cereus* and *Bacillus thuringiensis*, with leaf extracts exhibiting higher inhibitory effects. Notably, this study reports, for the first time, the antibacterial activity of *S. asper* against *B. cereus* and *B. thuringiensis*. The observed bioactivities are attributed to the presence of phenols, flavonoids, and saponins, which are recognized as plant-derived antimicrobial substances. The findings provide quantitative scientific validation of the traditional medicinal uses of *S. asper* and highlight its potential as a natural source of antioxidant and antibacterial agents for pharmaceutical and therapeutic applications.

Keywords: Antibacterial activity, phytochemicals, *B. cereus, B. thuringiensis*, zone of inhibition.

Introduction

Plants have acted as a fundamental repository of medicine since time immemorial. As reported by the World Health Organization (WHO, 2002), nearly 80% of the population in Africa, Asia, and Latin America continues to rely on traditional medicinal practices. The global reliance on natural substances has increased significantly in recent few decades due to their easy accessibility without prescription, low cost, and a widespread prevailing perception that herbal remedies provide greater safety and effectiveness more than synthetic drugs[1]. However, this perception is often misleading. Numerous reports of herbal medicines indicate that it may lead to adverse effects including potentially harmful interactions with conventional prescription drugs[2,3,4,5,6]. These concerns highlight the necessity of comprehensive scientific investigation to better understand the pharmacological properties, safety profiles, and therapeutic value of medicinal plants.

In North-East India, traditional medicine is deeply embedded within the cultural fabric of its diverse ethnic communities. This area is known as one of the biodiversity hotspots in India, is home to a significant number of plants and animals. About 500 plants taxa are estimated to be regularly utilized in native therapy[7,8]. Ethnobotanical research has revealed that many tribal communities rely on various medicinal plants for everyday healthcare routine. Utilizing them in various forms such as applying them topically as poultices and pastes, and consuming them in the form of juices, decoctions, or even food-based remedies like curries. Across the world, around 7,000 species are documented to possess pharmacological and nutritional significance, acting as reservoirs of essential phytochemicals and macro- and micronutrients[9]. Coupled with the ethnomedicinal strength and the heavy reliance on such resources, it becomes crucial to scientifically evaluate traditionally used plants to confirm their healing potential and ensure they are safe to use.

The *Streblus asper* Lour., is commonly known as the toothbrush tree, also a medicinally significant plant from the Moraceae family. This plant species is indigenous to several regions including India, Indonesia, Bangladesh, Laos, Myanmar, Nepal, Sri Lanka, Java and Vietnam. This tree is locally referred as “Saora” or “Soura” among Assamese

people. *S. asper* is a medium-sized tree characterized by milky latex, soft light-grey bark, rigid serrated leaves, and minute dioecious flowers. The species typically flowers from January to March and bears fruit from April to May[10]. Ethnomedicinal literature highlights the extensive therapeutic use of nearly all plant parts, including roots, bark, leaves, stems, fruits, and seeds, for treating various ailments[9,10]. Pharmacological studies have revealed that *S. asper* possesses antimicrobial, anti-inflammatory, antioxidant, antipyretic, antidiarrheal, antiplaque, and antidysenteric properties[11,12]. The plant is particularly rich in cardiac glycosides, triterpenoids, and phytosterols, which contribute to its wide therapeutic potential[13].

In the traditional practices of Assam, *S. asper* plays a multifaceted role. Seeds are used as deworming agents for both humans and livestock; tender branches serve as natural toothbrushes for maintaining oral hygiene; and leaf particulates homogenized with honey are consumed to alleviate digestive issues and haemorrhoids. Leaf pastes soaked overnight in water with rock sugar are traditionally used to relieve urinary irritation. This species holds significance because of its edible fruits, use as fodder, ornamental appeal, shade around ponds, durable timber, and its utility as a source of firewood. Given its diverse ethnobotanical applications and the growing scientific interest in its bioactive constituents, *S. asper* represents a promising yet underexplored medicinal resource. Therefore, the present study focuses on systematically evaluate the phytochemical constituents, antioxidant activity, and antimicrobial properties of *S. asper* leaves and branches, contributing to a deeper understanding of its therapeutic potential and validating its traditional uses.

MATERIALS AND METHODS

Preparation of aqueous extracts: Fresh leaves and small-sized branches of *S. asper* were collected from the outskirts of Kokrajhar town in Assam. The collected plant materials were initially cleaned, weighed, and thoroughly rinsed under running tap water to remove dust and debris. The collected samples were then shade-dried for about a week. After complete drying, they were reweighed to determine the moisture content. Subsequently, the dried samples were milled into fine powder using an electric blender. The calculation of moisture contentment was performed by using following formula:

$$\text{Moisture Content (\%)} = \text{Raw weight} - \text{Dry weight} \div \text{Raw weight} \times 100$$

The weight of the dry powder was measured and then stored in air-tight bottles. To prepare aqueous leaf extract, the method described Mbaebie et al (2012)[14] was followed. Samples were submitted for freeze drying/lyophilization at Guwahati Biotech Park, Amingaon, Guwahati, Assam. Lyophilization at -500C was carried out to get aqueous plant extract. Freshly prepared extracts were weighed and stored in a refrigerator for further use (Figure 1).

Quantitative phytochemical analysis and analysis of antioxidant activities of the extracts: Phytochemical constituents, including phenols, flavonoids, tannins, and saponins, along with antioxidant activity (DPPH radical scavenging, IC_{50}), were quantitatively estimated following standard referenced methods. Freeze-dried aqueous extracts were reconstituted in distilled water (1:1) to prepare the stock solution, which was subsequently diluted to obtain the required concentrations. All analyses were performed in triplicate (n = 3), and results were expressed as Mean ± SE.

Total Flavonoid Content (TFC): Total flavonoid content was estimated following the methods described by Woisky et al (1998) & Ordonez et al (2006)[15,16]. Quercetin is used as a reference chemical. Values were measured spectrophotometrically at 420 nm and expressed as µg quercetin equivalent (QE)/mg plant extract.

Total Phenol Content (TPhC): Total phenol content was estimated following the methods described by Ordonez et al (2006) & Singleton et al (1999)[16,17]. Gallic acid is used as a reference chemical. Values were measured spectrophotometrically at 760 nm and expressed as µg gallic acid equivalent (GAE)/mg plant extract.

Total Tannin Content (TTC): Total tannin content was estimated following the methods described by Katoch (2011)[18]. Tannic acid is used as a reference chemical. Values were measured spectrophotometrically at 700 nm and expressed as µg tannic acid equivalent (TAE)/mg plant extract.

Total Saponin Content (TSC): Total saponin content was estimated following the methods described by Goel et al (2012) & Le et al (2018)[19,20] using quillaja saponin as a reference chemical. Values were measured spectrophotometrically at 544 nm and expressed as µg quillaja saponin equivalent (QSE)/mg plant extract.

Anti-oxidant Activity Study: 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity study was performed by following the works of Mamta & Saxensa (2012) & Pant et al (2015)[21,22] with slight modification. Ascorbic acid was taken as a standard, and absorbance was measured at 517 nm in a spectrophotometer. The scavenging activity of plant extracts was calculated using this formula:

DPPH Scavenging Activity (%) ={[Abs control - Abs sample]/Abs control} ×100

Where, Abs control = Absorbance of DPPH in methanol

Abs sample = Absorbance of DPPH in plant extract

Culture of bacteria and antibacterial efficiency test: Three bacterial strains, *Bacillus thuringiensis, Bacillus subtilis*, and *Bacillus cereus*, were cultured aseptically on sterile nutrient agar plates (90 × 17 mm) and incubated at 37 °C for 48 hours. Liquid cultures were then prepared in nutrient broth (25 × 150 mm test tubes) and incubated at 37 °C for 48 hours in a shaking incubator. Aqueous extracts of *S. asper* were evaluated for antibacterial activity using the disc diffusion method. One gram of plant extract was dissolved in 1 ml DMSO to obtain a stock solution of 500 mg/ml, from which three serial dilutions (10^{-1}, 10^{-2}, 10^{-3}) were prepared. Nutrient agar plates were inoculated with 250 µl of each bacterial suspension and spread uniformly using a sterile L-spreader. Sterile discs (HiMedia) were impregnated with the respective extract concentrations and placed on the inoculated plates along with a kanamycin disc (30 µg) as the positive control. Plates were incubated at 37 °C for 48 hours, and the zones of inhibition were measured in millimetre using an antibiotic zone scale (HiMedia).

Statistical analysis: Statistical analyses were performed using the mean values and standard errors (n = 3), and independent t-tests were applied to determine significance at $p \leq 0.05$.

RESULTS

The aqueous leaf and branch extracts of *S. asper*, analyzed using the above-mentioned methods, exhibited varying levels of total phenolic, flavonoid, tannin, and saponin contents, along with antioxidant activity and moisture content. Both extracts showed appreciable amounts of these phytochemical constituents and antioxidant potential (Figure 2). However, the quantitative values for all measured parameters were consistently higher in the leaf extract compared to the branch extract. Statistical analysis confirmed that all parameters differed significantly between the leaf and branch extracts ($p \leq 0.05$) (Table 1).

The aqueous leaf and branch extracts of *S. asper* exhibited notable antibacterial activity against both *B. thuringiensis* and *B. cereus*. Against *B. thuringiensis*, the aqueous leaf extract showed the highest inhibitory activity at the standard concentration (500 mg/ml), followed by progressively reduced activity at dilutions of 10^{-1}, 10^{-2}, and 10^{-3}. Although both plant extracts were effective, Kanamycin produced a larger zone of inhibition than the herbal extracts. A clear concentration-dependent variation in antibacterial activity was observed, with the aqueous leaf extract consistently producing larger inhibition zones than the branch extract across all tested concentrations (Figure 3).

Similarly, the aqueous leaf extract demonstrated the highest antibacterial activity against *B. cereus* at the standard concentration when compared with the branch extract. Kanamycin again exhibited superior inhibitory effects relative to both plant extracts. Notably, the antibacterial efficacy of the aqueous leaf extract against *B. cereus* was greater at all tested concentrations (standard to 10^{-3}) than its activity against *B. thuringiensis*. The aqueous branch extract also showed stronger inhibition of *B. cereus* than *B. thuringiensis* at all concentrations (Figure 4). These results indicate a comparatively higher antibacterial potential of both aqueous extracts of *S. asper* against *B. cereus*.

Statistical analysis revealed significant differences among the inhibition zones produced by the standard concentration, 10^{-1}, and 10^{-2} dilutions of the aqueous leaf and branch extracts against *B. thuringiensis* (Table 2). In contrast, no statistically significant differences were observed among the treated concentrations of the aqueous leaf and branch extracts against *B. cereus* (Table 3). The differences in the diameters of inhibition zones produced by Kanamycin and the aqueous leaf extract, as well as by Kanamycin and the aqueous branch extract, against *B. thuringiensis* were statistically significant (Table 4). On the other hand, in the case of *B. cereus*, statistically significant differences in inhibition zone diameters were observed between Kanamycin and both the aqueous leaf and branch extracts at the 10^{-1}, 10^{-2}, and 10^{-3} dilutions. At the standard concentration (500 mg/ml), the difference between Kanamycin and the plant extracts was not statistically significant (Table 5).

DISCUSSION

Phytochemicals are natural bioactive compounds produced by plants, where they provide crucial defensive roles against environmental stressors, pathogens, and herbivores. Beyond their ecological significance, these compounds exert a wide range of beneficial effects in humans and animals, including anti-inflammatory, antioxidant, antidiabetic, antifungal, antibacterial, and anti-obesity activities[23].

In this current investigation, quantitative phytochemical analysis of *S. asper* demonstrated a rich phytochemical profile in the aqueous extracts of both leaves and branches. Although both plant parts contained appreciable levels of phytoconstituents, the leaf extract consistently exhibited higher concentrations of most phytochemicals compared to the

branch extract, except for the total saponin content of the branch (67.12 ± 0.428 µg/mg). Among the analyzed compounds, phenolic content was predominant (87.41 ± 0.839 µg/mg in leaves and 80.91 ± 0.727 µg/mg in branches), followed by flavonoids (84.64 ± 0.979 µg/mg in leaves and 79.83 ± 0.555 µg/mg in branches) and tannins (83.91 ± 0.464 µg/mg in leaves and 78.34 ± 0.464 µg/mg in branches). These results provide strong scientific evidence supporting the broad therapeutic potential of *S. asper* (Figure 2).

Previous studies have reported the presence of diverse phytochemicals, such as alkaloids, saponins, reducing sugars, terpenoids, glycosides, phenols, ketones, flavonoids, carbohydrates, triterpenoids, phytosterols, tannins, coumarins, anthocyanins, cardiac glycosides, lignans, neolignans, streblin, cerotic acid, and octacosanoic acid, in different solvent extracts (methanol, ethanol, aqueous, petroleum ether, and hydroalcoholic) prepared from different parts of plant, including leaves, roots, and stem bark of *S. asper*[9,24,25]. However, earlier literature largely focused on qualitative screening, with limited quantitative evaluation of these bioactive compounds. In this context, the present study fills an important knowledge gap by providing quantitative data on key phytochemicals, namely phenols, flavonoids, tannins, and saponins, which are known to play significant roles in physiological, metabolic, and immunological processes.

The study further highlights the antioxidant potential of aqueous leaf and branch extracts of *S. asper*. DPPH radical scavenging assays revealed antioxidant activities of 55.05 ± 0.467% in leaf extracts and 45.86 ± 0.664% in branch extracts (Figure 2). Supporting these findings, it was reported in [9] that a DPPH IC_{50} value of 263.84 ± 0.14 µg/ml in hydroalcoholic leaf extracts of *S. asper*, enhancing the plant's strong antioxidant potential. Differences in solvent polarity during extraction constitute the primary factor underlying inconsistencies in phytochemical profiles and antioxidant potential across studies[26].

Solvent selection plays an essential role in determining extraction efficiency, as it influences compound solubility, selectivity, safety, yield, extraction time, and temperature[27]. Over time, in phytochemical extraction, the polar solvents show the highest effectiveness. Water stands out as the most economical, environmentally friendly, and safest solvent among them[28]. Aqueous extraction may significantly result in lower concentrations of bioactive compounds compared to alcoholic solvents, and this could also be the reason why it is found to have low antioxidant activity compared to hydroalcoholic extracts[29]. Their study showed that freeze-dried leaf extracts of *S. asper* effectively protected against oxidative damage caused by free radicals. This observation is particularly relevant to the present work, as the aqueous leaf and branch extracts were prepared using a freeze-drying technique, which likely contributed to the appreciable phytochemical yield and antioxidant activity observed.

The novelty of this current investigation is particularly significant from an ethnomedicinal validation perspective. Almost every part of *S. asper* has been extensively used in Ayurveda and Indian folk medicine for centuries. Owing to its rich content of cardiac glycosides and other phytochemicals, the plant has been reported to possess antibacterial[30,31,32], anti-inflammatory[33,34], antioxidant[29,30,35,36,37], antimicrobial[36,38] antimplantation[25] and anticancer[39] activities. Additionally, various morphological structures of plant are utilized traditionally to treat ailments such as dysentery, constipation, mouth ulcers, gum pain, eczema, itching, scabies, ringworm, tooth bleeding, amenorrhea, acne, fever, toothache, gingivitis, filariasis, epilepsy, epistaxis, piles, and stomach disorders[10,24,25,40]. Researchers have also suggested the development of *S. asper* leaves as oral dental products and natural contraceptive agents. In North-East India, particularly in Assam, people have traditionally used leaves to treat urinary disorders, while seeds and branches serve as natural deworming agents and oral hygiene tools, respectively.

The present investigation scientifically validates these traditional practices by demonstrating the presence of bioactive phytochemicals, including phenols, flavonoids, tannins, and saponins, as well as strong antioxidant and antibacterial activities in both leaves and branches.

The study revealed that aqueous extracts of *S. asper* exhibited greater inhibitory effects against *B. cereus* than against *B. thuringiensis*. Leaf extracts produced larger inhibition zones against *B. thuringiensis* (15.5 ± 0.577 mm, 13.7 ± 0.166 mm, 12.6 ± 0.333 mm, and 10.1 ± 0.440 mm) compared to branch extracts (12.3 ± 1.301 mm, 10.1 ± 0.167 mm, 10.6 ± 0.166 mm, and 10.6 ± 0.333 mm) (Figure 3). A similar trend was observed against *B. cereus*, where leaf extracts demonstrated stronger antibacterial activity (27.5 ± 0.5 mm, 22.6 ± 0.881 mm, 15.3 ± 0.166 mm, and 11.6 ± 0.833 mm) than branch extracts (24.6 ± 1.166 mm, 19.8 ± 1.452 mm, 13.3 ± 1.092 mm, and 10.6 ± 0.107 mm) (Figure 4).

S. asper is already recognized as a potent antibacterial plant species in earlier studies[9,10,24,36]. Ethanolic acid fractions of leaf extracts have shown strong antibacterial activity against Gram-positive bacteria like Staphylococcus aureus as well as *Bacillus subtilis*, with an MIC of 125 µg/ml, while showing limited efficacy against Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Further it was reported that potent antibacterial effects of hydroalcoholic leaf extracts against *E. coli* and *S. aureus*, with minimum inhibitory concentration values of 60 µl (E. coli: 1.2 cm; S. aureus: 1.2 cm) and maximum inhibitory concentrations of 120 µl (E. coli: 1.5 cm, S. aureus: 1.9 cm)[9]. The findings of the present study are largely consistent with these reports. Importantly, this work reports, for the first

time, the antibacterial activity of *S. asper* against *B. thuringiensis* and *B. cereus*, highlighting the versatility of the species in terms of antibacterial potential against a wide range of bacterial species. These antibacterial effects can be attributed to the presence of phenols, flavonoids, and saponins; phytochemicals that are widely recognised for their antimicrobial properties[41,42,43]. Such bioactive molecules collectively constitute Plant-Derived Antimicrobial Substances (PDAMs), which play a significant role in preventing pathogenic bacterial contamination[44,45].

The combined evidence of phytochemical richness, antioxidant capacity, and antibacterial activity underscores the medicinal significance of *S. asper* and provides robust scientific validation of its long-standing use in traditional healthcare systems.

CONCLUSION

The present study demonstrates that *S. asper* is a rich reservoir of bioactive phytochemicals, particularly phenols, flavonoids, tannins, and saponins, with aqueous leaf extracts showing higher concentrations and stronger biological activities than branch extracts. The appreciable antioxidant and antibacterial activities observed, especially against *B. cereus* and *B. thuringiensis*, highlight the therapeutic potential of this species. Importantly, this study provides quantitative phytochemical data and reports antibacterial activity against *B. cereus* and *B. thuringiensis* for the first time, thereby filling critical gaps in existing literature. The findings scientifically validate the traditional and ethnomedicinal uses of *S. asper* and support its potential development as a natural source of antioxidant and antimicrobial agents for pharmaceutical and healthcare applications.

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Tables

Table 1: Summary of the statistical significance ($p \leq 0.05$)

Parameters	Standard Error		t-value	p-value
	Leaf	Branch		
Phenol	0.839	0.727	5.855	0.0042*
Flavonoid	0.979	0.555	4.275	0.0129*
Tannin	0.464	0.464	8.488	0.0010*
Saponin	0.476	0.428	-26.276	0.000012*
DPPH (IC_{50})	0.467	0.664	11.321	0.000347*
Moisture (%)	0.045	0.032	140.172	1.55×10^{-8}*

'*' denotes statistical significance between leaf and branch extracts at $p \leq 0.05$

Table 2: Comparative Summary of the statistical significance ($p \leq 0.05$) in terms of zone of inhibition by aqueous extracts of leaf and branch against *B. thuringiensis*

Concentrations	Standard error		t-value	p-value
	Leaf	Branch		
Standard (500 mg/ml)	0.577	1.301	4.302	0.048^{*}
10^{-1}	0.166	0.167	4.302	0.006^{*}
10^{-2}	0.333	0.166	4.302	0.020^{*}
10^{-3}	0.440	0.333	4.302	1^{NS}

'*' denotes statistical significance between leaf and branch extracts at $p \leq 0.05$; 'NS' denotes not significant

Table 3: Comparative Summary of the statistical significance ($p \leq 0.05$) in terms of zone of inhibition by aqueous extracts of leaf and branch against *B. cereus*

Concentrations	Standard error		t-value	p-value
	Leaf	Branch		
Standard (500 mg/ml)	0.5	1.166	4.302	0.170^{NS}

10^{-1}	0.881	1.452	4.302	0.072^{NS}
10^{-2}	0.166	1.092	4.302	0.252^{NS}
10^{-3}	0.833	0.167	4.302	0.188^{NS}

'NS' denotes not significant

Table 4: Comparative Summary of the statistical significance ($p \leq 0.05$) in terms of zone of inhibition by Kanamycin, aqueous extracts of leaf and branch against B. thurigiensis

Concentrations of aqueous extracts	Kanamycin and Aqueous Leaf Extract		Kanamycin and Aqueous Branch Extract		Standard Error of Kanamycin
	p-value	t-value	p-value	t-value	
Standard (500 mg/ml)	0.001^{*}	4.302	0.004^{*}	4.302	0.289
10^{-1}	$9.8E\text{-}05^{*}$	4.302	0.002^{*}	4.302	
10^{-2}	0.001^{*}	4.302	0.004^{*}	4.302	
10^{-3}	0.002^{*}	4.302	0.009^{*}	4.302	

'*' denotes statistical significance between leaf and branch extracts at $p \leq 0.05$

Table 5: Comparative Summary of the statistical significance ($p \leq 0.05$) in terms of zone of inhibition by Kanamycin, aqueous extracts of leaf and branch against *B. cereus*

Concentrations of aqueous extracts	Kanamycin and Aqueous Leaf Extract		Kanamycin and Aqueous Branch Extract		Standard Error of Kanamycin
	p-value	t-value	p-value	t-value	
Standard (500 mg/ml)	0.212NS	4.302	0.068NS	4.302	0.667
10-1	0.026*	4.302	0.024*	4.302	
10-2	0.002*	4.302	0.004*	4.302	
10-3	0.005*	4.302	0.001*	4.302	

'*' denotes statistical significance between leaf and branch extracts at $p \leq 0.05$; 'NS' denotes not significant

Figures

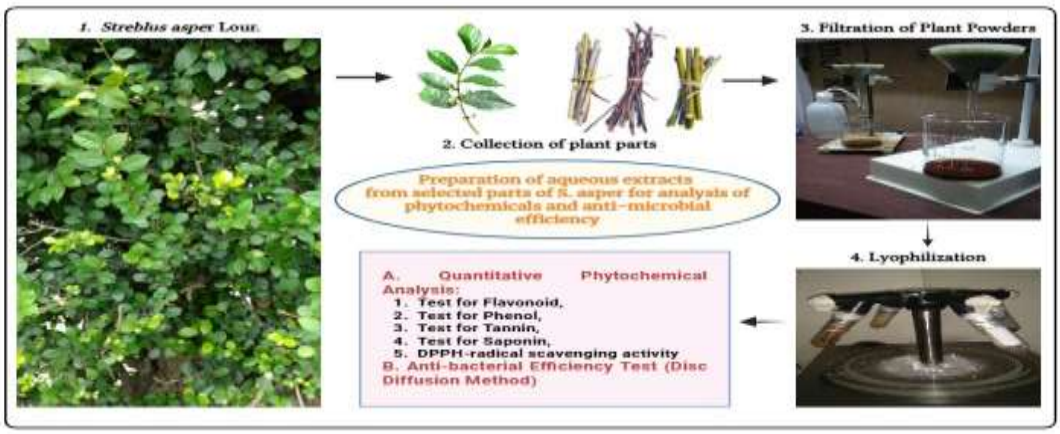


Figure 1: Processes involved in the preparation of extracts and analysis of different parameters for ethnopharmacological validation of *S. asper*.

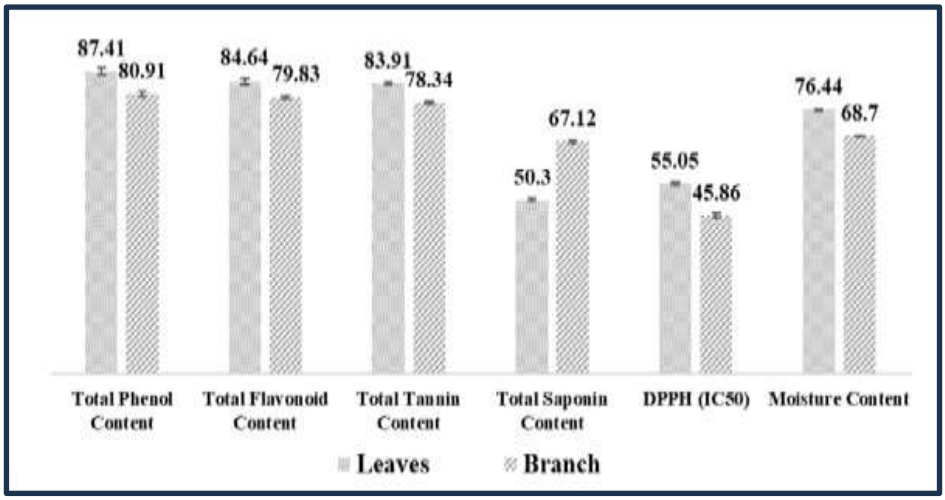


Figure 2: Quantitative phytochemical analysis of the aqueous extracts of *S. asper*

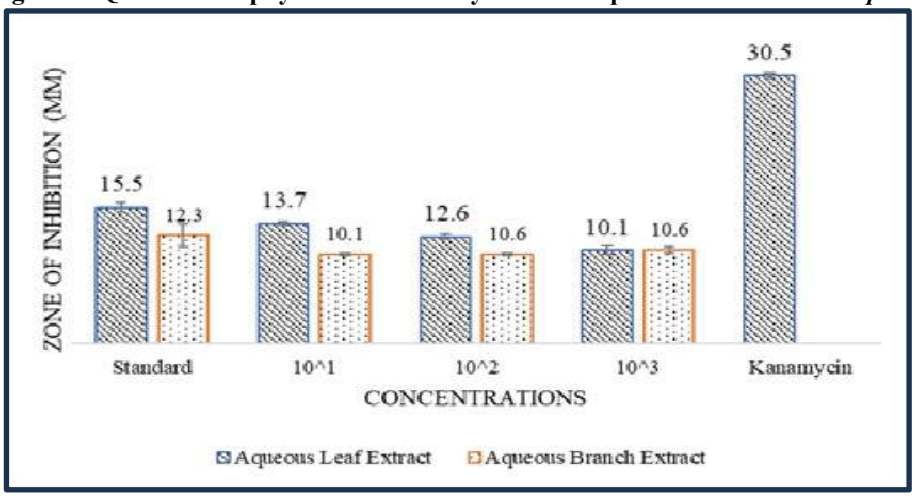


Figure 3: Zone of inhibition against *B. thuringiensis* by aqueous leaf and branch extracts of *S. asper* and Kanamycin

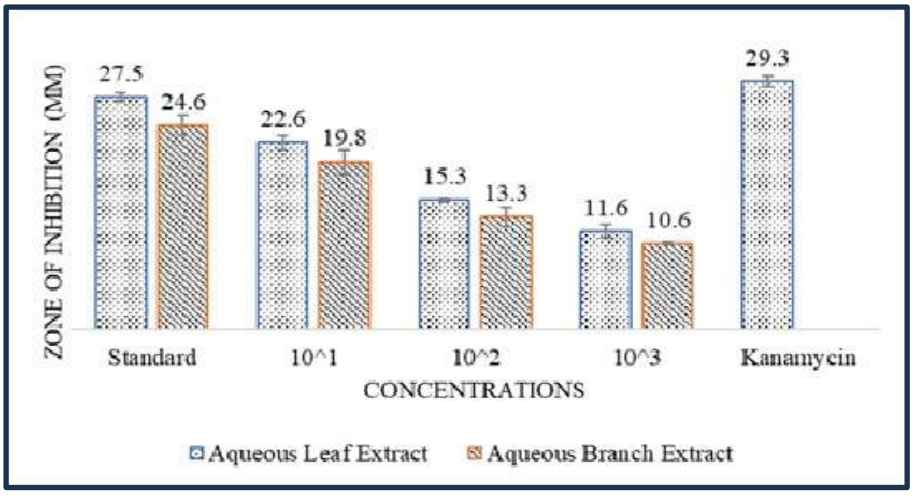
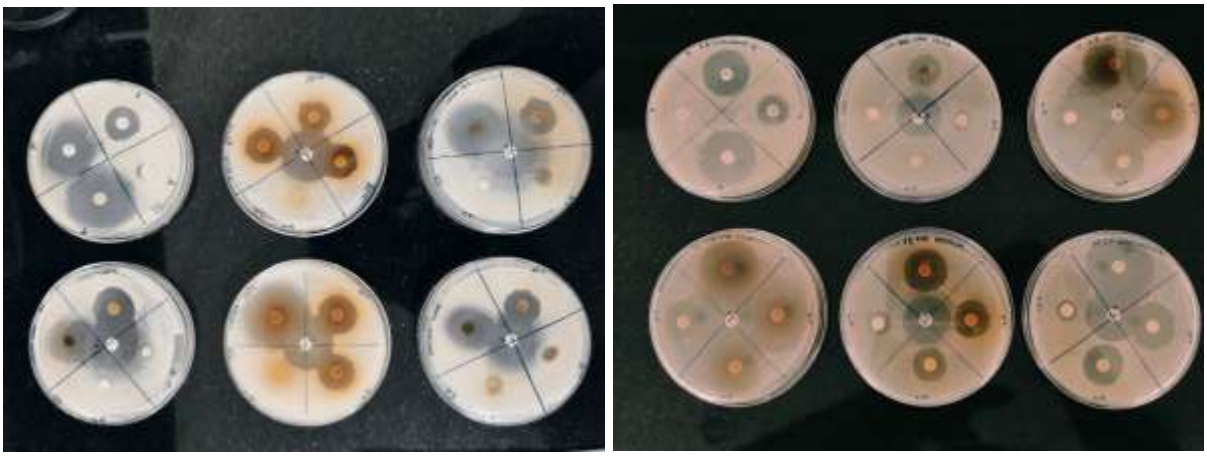


Figure 4: Zone of inhibition against *B. cereus* by aqueous leaf and branch extracts of *S. asper* and Kanamycin



A **B**

Figure 5 (A-B): Zones of inhibition produced by aqueous leaf and branch extracts of *S. asper*. A. Zone of inhibition against *B. thuringiensis*; B. Zone of inhibition against *B. cereus*.