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Madan Mohan Pandey
Pharmacognosy, Phytochemistry and Product Development Division, CSIR-National Botanical Research Institute, Lucknow, India

Subha Rastogi
Pharmacognosy, Phytochemistry and Product Development Division, CSIR-National Botanical Research Institute, Lucknow, India

Corresponding Author:
Subha Rastogi
Pharmacognosy, Phytochemistry and Product Development Division, CSIR-National Botanical Research Institute, Lucknow, India

Streblus asper: A phytochemical, ethnopharmacological and pharmacological research update

Madan Mohan Pandey and Subha Rastogi

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Abstract
Streblus asper Lour (Family: Moraceae), known as the toothbrush tree, is an important medicinal plant. It has been used as medicine in different parts of the world, especially in the South Asian countries, for the treatment of various diseases such as dysentery, toothache, gingivitis, filariasis, epilepsy, epistaxis, piles and stomachache. A large number of cardiac glycosides have previously been reported from the root bark of this tree. The present review provides an update on the recent research and the new reports of ethnomedicinal claims, phytochemical investigations and the advances made in pharmacological evaluations of *S. asper* during the period 2006-2021 in order to explore its potential for future research.

Keywords: *Streblus asper*, shakhotaka, lignans and neolignans, anticancer, orodental

Introduction
Streblus asper Lour (Family: Moraceae), an important medicinal plant of the 'Fig' family, is geographically distributed mostly in tropical and subtropical Asia. It is included in the Ayurvedic Pharmacopoeia of India [1]. Besides, certain monographs on medicinal plants also describe *S. asper* [2]. Almost every part of *S. asper* has been used extensively in Ayurveda and folk medicine for centuries [3]. *S. asper* is a rich source of cardiac glycosides and more than 20 cardiac glycosides have been isolated by Reichstein and co-workers from the root bark of *S.* asper [4]. *S. asper* has proven properties like anti-inflammatory, antioxidant, antimicrobial, antibiofilm and anticancer activity and different parts of the plant have been used for the treatment of various diseases such as dysentery, relief of toothache, antigingivitis, filariasis, epilepsy, epistaxis, piles and stomachache. Findings have suggested that there is a potential for developing *S. asper* as a natural oral hygiene product [5]. Earlier we had compiled a comprehensive review of *S. asper* [4] that covered its traditional and folk medicinal uses, phytochemistry and pharmacology. The objective of the present article is to provide an update on the recent research on the new reports of its ethnomedicinal claims, phytochemical investigations and the advances made in pharmacological evaluations of this important ethnomedicinal plant since during the period 2006-2021.

Ethnomedicinal/Traditional uses
The available literature and information show that different parts of *S. asper* have traditionally been used as medicine in different parts of the world, including the Indian Traditional System of Medicine [6]. The various traditional uses, local name, as well as the mode of preparation or use that have been reported [7-19] have been presented in Table 1.

Phytochemistry
Streblus asper is a rich source of cardiac glycosides. Earlier, Reichstein and co-workers had isolated and characterized a large number of cardiac glycosides from the root bark of *S. asper*. Also, earlier, the main focus was on the cardiac glycosides present in this species. However, during the past few years several known as well as novel phytoconstituents belonging to different groups of compounds have been isolated and identified from *S. asper*. Most of the lignans and neolignans are novel compounds and reported for the first time from *S. asper*. Table 2 enlists the different new phytoconstituents that have been reported from *S. asper* [20-27], as well as their basic phytochemical groups, whereas their corresponding structures have been shown in Figures 1-4. A novel serine protease, Streblin, has also been isolated from *S. asper* [28].

Table 1. Traditional uses of *S. asper*

S. No.	Country	Area	Local name	Plant part	Traditional use	Mode of preparation/ use	Reference
1	Bangladesh	Thanchi, Bandarban Hill Tracts	-	Root	Amenorrhea	Amenorrhea: 1–2 spoonful of root juice is given 3–4 times daily.	[7]
					Toothache	Toothache: Powder of root and ash of jackfruit leaf is used together externally.	
					Fever	Fever:1spoonful of root juice is taken 2–3 times daily	
2	China	Limu Mountains of Hainan Island	Kēnpān	Root	Stomach-ache	Decoction is given orally	[8]
3	India	Assam	Saura	Bark, Branch	Teeth are protected from microbes	The teeth are brushed with the cut branches	[9]
		Southern Assam	Shera	Bark	Digestive system disorder	Infusion is used	[10]
		Unakoti District, Tripura, (Chakma and Halam tribes)	Sheora	Leaf	Diabetes	Decoction is used	[11]
		Tripura (Reang people)	Salua	Leaf	Dysentery	Juice	[12]
		Similipal Biosphere Reserve and Mayurbhanj district, Odisha	Sahada	Root powder	Menstrual irregularities	-	[13]
				Bark	Anti-skin parasite, anticancer, anthelmintic		[14]
		Three western districts of West Bengal	Seowra	Leaves	animal bites and insect stings	4–5 Leaves are taken and rubbed by hand to extract juice and the juice is given on the stinging area	[15]
		Keonjhar district, Odisha	Sahada	twigs	toothache	Regular brushing of teeth by fresh tender twigs	[16]
		Udalguri district, Assam	Soura	stem	anthelmintic	Raw	[17]
4	Pakistan	South Waziristan Agency and Bajaur Agency, Federally Administrated Tribal Areas (FATA)	Tor tooth	Fruit	Ethnoveterinary use - as a cooling agent for cow, goat	Oral- 12 2 kg fresh fruit of the plant along little amount of water are crushed and then juice is extracted which is given orally to the animal for the production of cooling effect	[18]
5	Thailand	Three southern border provinces - Pattani, Yala and Narathiwat		Bark Sap	Dental Caries	Chopped bark and a small amount of salt is boiled with water. Keep drug in the mouth for 1– 2 min	[19]
				juice		Unprocessed- Apply to the gum at bedtime	

Table 2: Phytochemical constituents of *S. asper*

S. No.	Phytochemical groups	Compounds	References
1	Lignans and Neolignans	magnolignan A-2-O-β-D-glucopyranoside (1)	[20]
		streblusligna nol (2)	
		magnolignan A (3)	
		magnolol (4)	
		magnaldehyde D (5)	
		(7'S,8'S)-trans-streblusol A (6)	
		(7'R,8'S)-erythro-streblusol B (7)	[21]
		(7'S,8'S)-threo-streblusol B (8)	
		8'R-streblusol C (9)	
		(8R,8'R)-streblusol D (10)	
		streblusquinone (11)	
		streblusol E (12)	
		9-β-xylopyranosyl-isolariciresinol (13)	
		(7R,8S,7'R,8'S)-erythro-streblusligna nol H (14) honokiol	[22]
		erythro-streblusligna nol (15)	
		threo-7′-methoxyl streblusligna nol (16)	
		erythro-7′-methoxyl streblusligna nol (17)	[23]
		(7'R,8'S,7''R,8''S)-erythro-streblusligna nol G (18)	
		streblusligna nol F (19)	

		isostrebluslignanaldehyde (20)	[24]
		isomagnaldehyde (21)	
		isomagnolol (22)	
		isolariciresinol (23)	
		(7′R,8′S)-4,4'-dimethoxy-strebluslignanol (24)	
		3'-hydroxy-isostrebluslignaldehyde (25)	
		3,3'-methylene-bis(4-hydroxybenzaldehyde) (26)	
		4-methoxy-isomagnaldehyde (27)	[25]
2	Triterpenoids and Sterols	ursolic acid (28)	[23]
		lup-20(29)-en-3β-olyl octadec-9′-enoate (1) (Lupeol oleate) (29)	
		lupeol linoleate (30)	
		stigmast-5-en-3β-olyl-26-oic acid-3β-hexadecanoate (Streblusteryl palmitate) (31)	[26]
		stigmasterol palmitate (32)	
3	Cardiac glycosides	(+)-19-hydroxykamaloside (33)	[27]
		(+)-5-hydroxyasperoside (34)	
		(+)-3'-de-O-methylkamaloside (35)	
		(+)-3-O-β-D-fucopyranosylperiplogenin (36)	
4	Miscellaneous	6-hydroxyl-7-methoxyl-coumarin (37)	[23]
		cerotic acid (38)	
		octacosanoic acid (39)	[26]

Pharmacology

4.1 Antioxidant activity

Several studies have been carried out on the leaves of *S. asper* to evaluate their antioxidant potential [29-33].

The petroleum ether leaf extract of *S. as per* (SAPE) was evaluated for its *in-vivo* antioxidant activity in diabetic rats. It increased the levels of enzymatic and non-enzymatic antioxidant entities along with reduced MDA levels. It is evident from the present study that SAPE was able to prevent and improve the deteriorating antioxidants parameters in the tissues of the treated diabetic animals [29].

In vitro antioxidant activity of the methanolic extract of the leaves of *S. asper* was determined using DPPH and H_2O_2 assay models and IC_{50} determined. Well known antioxidants ascorbic acid and tocopherol were used for comparison [30].

In another study, the leaves of *S. asper* were oven dried as well as freeze dried and the DPPH assay was used to evaluate the antioxidant potential of their aqueous and ethanolic extracts. Marked differences in the antioxidant activity were observed in the oven dried and freeze-dried samples. Extracts obtained from the freeze-dried samples exhibited a better free radical scavenging activity as compared to those obtained from oven dried samples. The studies also showed that the phenolic and flavonoid contents of the 70% ethanolic extract obtained from the freeze-dried samples were higher as compared to the others. The findings of the study led to the conclusion that the aqueous extracts obtained from the freeze-dried leaves had a potential to be used to prevent oxidative damage caused by free radicals [31].

Studies were carried out to determine the antioxidant activity of the neutral, acidic, and basic fractions obtained from the ethanolic extract of the leaves of *S. asper* using the DPPH and ABTS assays. The ABTS radicals were more affected as compared to the DPPH radicals. Although a concentration dependent free radical scavenging effect was observed for all the extracts and fractions, the antioxidant activity exhibited by acidic fraction > basic fraction > neutral fraction[32].

Besides the leaves, the antioxidant activity of the stem bark was also evaluated. *In vitro* experiments were conducted using DPPH, nitric oxide, hydroxyl radical, peroxynitrite and superoxide radicals and in all the models a concentration dependent antioxidant activity was observed. The IC_{50} values against all the free radicals were also determined [33].

4.2 Hypoglycemic/ Antidiabetic activity

The leaves, stem bark and root extracts of *S. asper* have been subjected to various studies using streptozotocin as well as alloxan induced diabetic animal models in order to ascertain their hypoglycemic or antidiabetic potential [29, 30, 34-36].

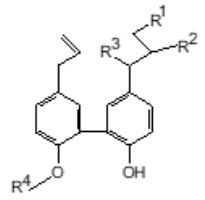
The petroleum ether leaf extract of *S. asper* (SAPE) was evaluated for its hypoglycemic property. In the diabetic animals, the blood glucose level was found to be significantly reduced after 4 hours of SAPE treatment ($p<0.01$,). Similarly, after 30 days blood glucose levels of the SAPE treated diabetic animals were at par with the glibenclamide treated diabetic rats ($p<0.001$). Also, a significant reduction in the loss of body weight in SAPE treated animals was observed ($p<0.001$) whereas, in comparison to the control animals, the animals treated with glibenclamide did not show any significant change in their body weight. Thus, SAPE treatment significantly prevented the loss of body weight of diabetic animals ($p<0.05$) [29].

The methanolic leaf extract of *S. asper*, when tested at 200mg and 400mg per kg bodyweight, significantly reduced the blood glucose levels in hyperglycemic rats as compared to the control. The reduction was found to be dose dependent. The antihyperglycemic effects were seen after 1 h of administering the extract [30].

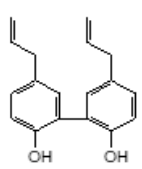
The petroleum ether extract of the roots of *S. asper* was tested for antidiabetic activity. When tested a dose level of 250 mg per kg bodyweight, it showed significant protection and lowering of the blood glucose levels to normal [34].

In another study the stem bark of *S. asper* was extracted with petroleum ether and this extract was evaluated for its antidiabetic activity. Dose dependent studies at three different doses of 100, 250 and 500 mg per kg bodyweight orally were carried out in streptozotocin (STZ)-induced diabetic rats. Glibenclamide was used as reference drug. Simultaneously, biochemical parameters and serum lipid profiles were also monitored. The results obtained showed that the administration of the petroleum ether extract led to the normalization of the blood glucose levels as well the different biochemical parameters of the serum [35].

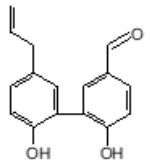
Studies conducted by using different extracts of the leaves and bark of *S. asper* in alloxan induced diabetic rats showed that significant antidiabetic effects were observed when administered at 300 mg/kg bw for 3 weeks orally [36].



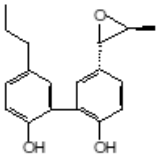
R^1=R^2= OH, R^3= glucose; Magnolignan A-2-*O*-*β*-D-Glucopyranoside (1)
R^1=R^4= H, R^2=R^3= OH; Streblus lignanol (2)
R^1=R^2= OH, R^3=R^4= H; Magnolignan A (3)



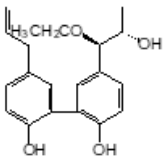
Magnolol (4)



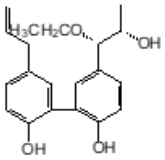
Magnaldehyde D (5)



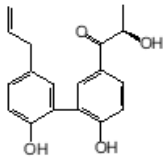
(7'S, 8'S)-*Trans*-streblusol A (6)



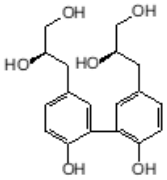
(7'R, 8'S)-*erythro*-streblusol B (7)



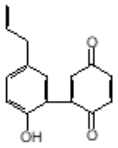
(7'S, 8'S)-*threo*-streblusol B (8)



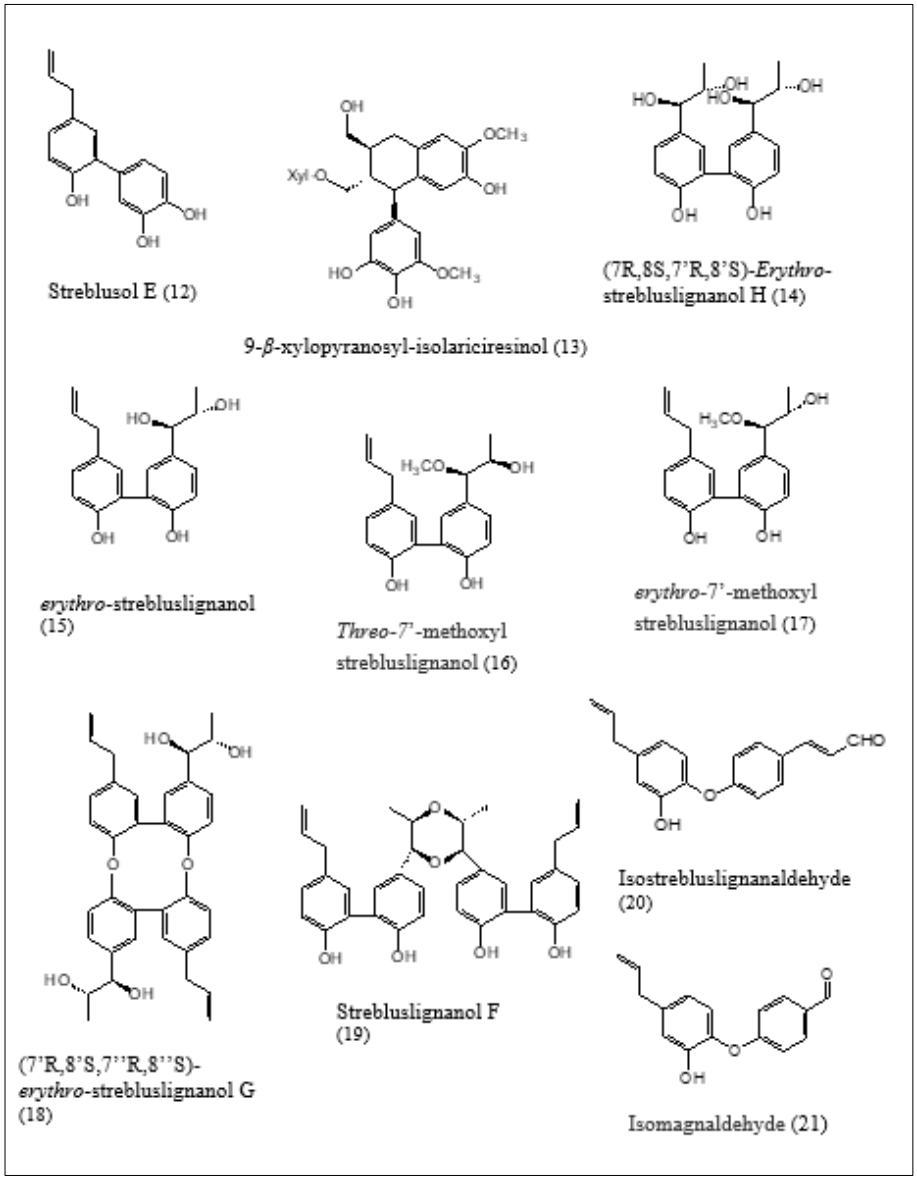
8'R-streblusol C (9)



(8R,8'R)-streblusol D (10)



Streblusquinone (11)



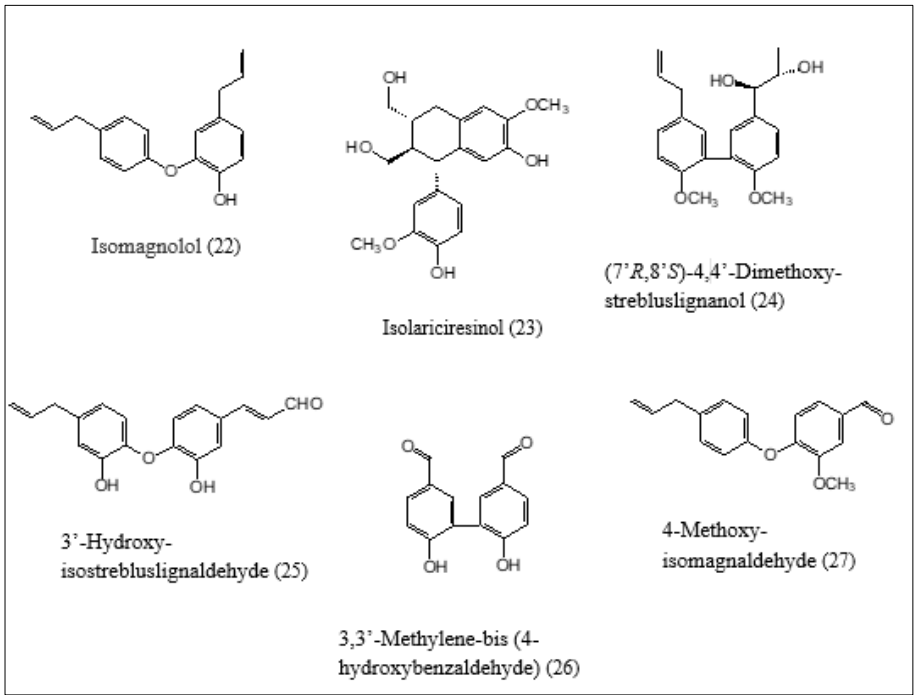


Fig 1: Structures of lignans and neolignans isolated from *S. asper*

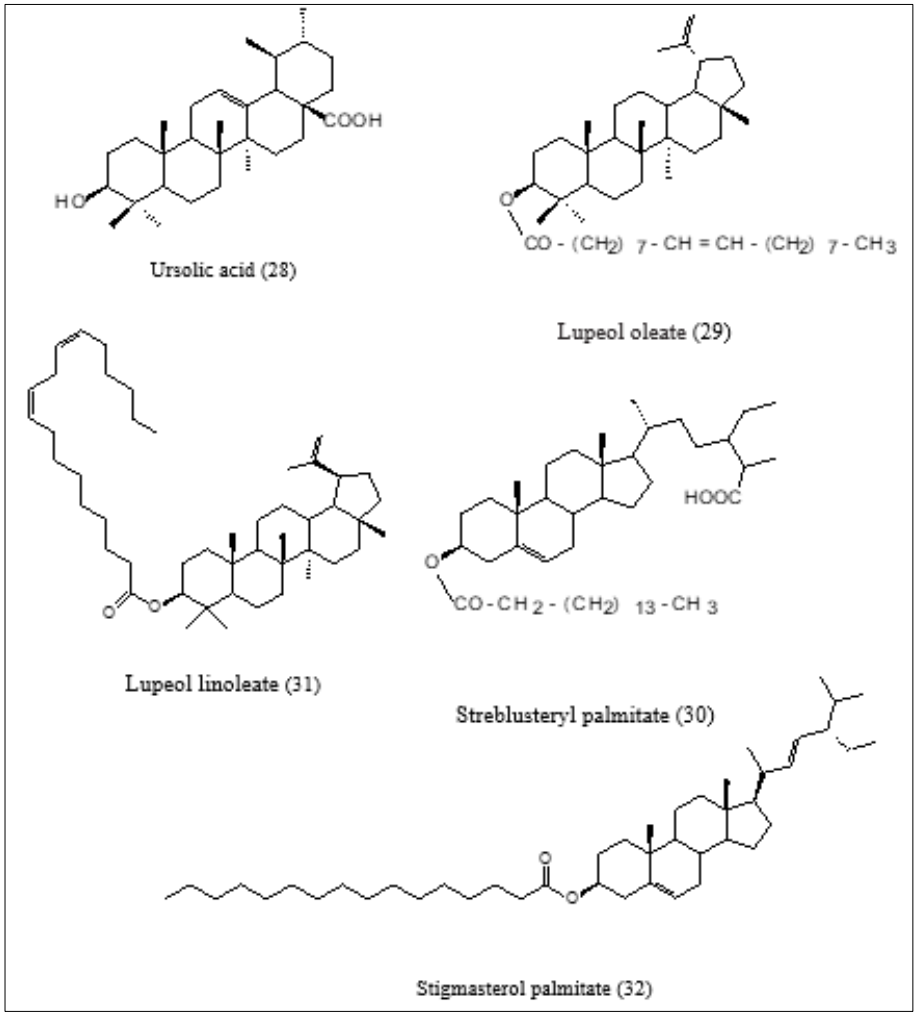


Fig 2: Structures of triterpenoids and sterols isolated from *S. asper*

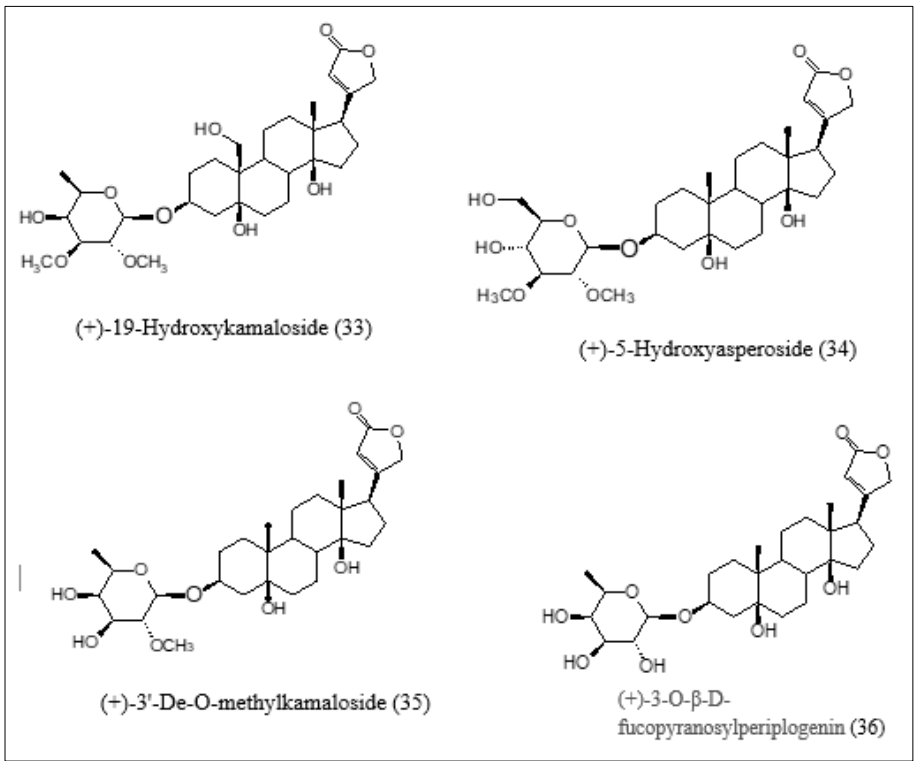


Fig 3: Structures of cardiac glycosides isolated from *S. asper*

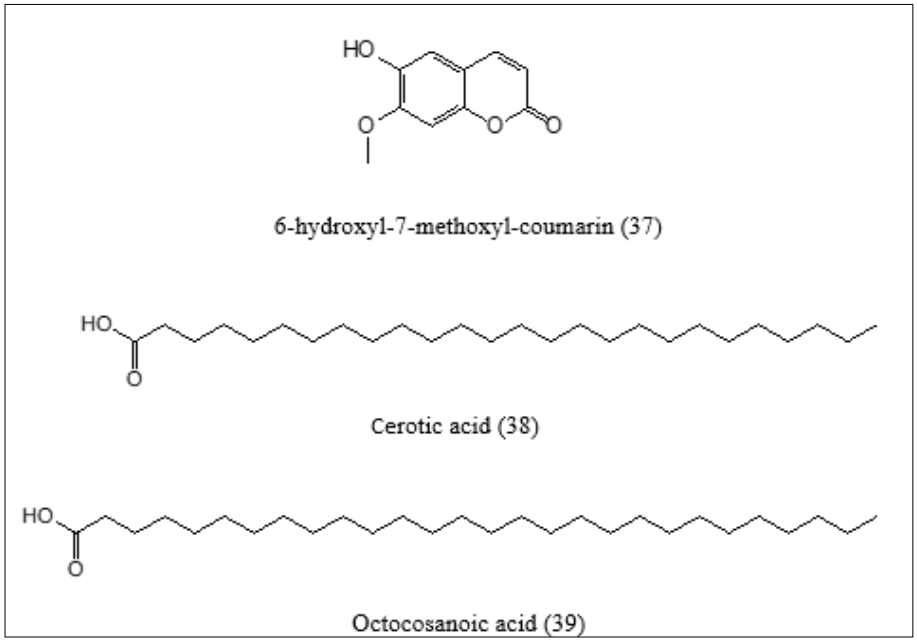


Fig 4: Miscellaneous compounds isolated from *S. asper*

4.3 Anti-inflammatory activity

The leaves of *S. asper* were studied for their anti-inflammatory activity in *in vitro* as well as *in vivo* models [37, 38].

The methanolic extract of *S. asper* leaves was assessed for its membrane stabilising activity in order to determine its anti-inflammatory activity *in-vitro*. Due to the similarities between the membranes of the human RBCs and the lysosomal membrane components, hypotonicity induced and heat induced haemolysis models using erythrocytes were used to assess the membrane stabilizing activity of the extract. The greater the inhibition of membrane lysis, the better the anti-inflammatory activity. Ethyl salicylic acid was used as reference drug for comparison. Tests were conducted using three different doses (200, 400 and 800 µg/ml of the extract). Significant inhibition in haemolysis was observed indicating anti-inflammatory nature of the extract [37].

The anti-inflammatory activity of *S. asper* leaf extract was assessed using carrageenan induced rat paw edema model. The plant extract exhibited a dose dependent manner of action. At the maximum concentration of SAE (500mg/kg body weight), the % inhibition of paw edema was comparable to the standard non-steroidal anti-inflammatory drug diclofenac. In order to investigate the possible mode of the anti-inflammatory action of SAE, RAW 264.7 macrophage cells were induced with LPS, then COX-2, COX-1 and iNOS mRNA expressions were determined by RT-PCR. The results demonstrated that SAE significantly suppressed the LPS-induced expression of COX-2 and iNOS mRNA in a dose–response manner [38].

4.4 Insecticidal

Studies aimed at assessing the insecticidal potential of the methanolic extract of *S. as per* on the stored grain pest, *Trogoderma granarium* Everts were carried out. It was observed that when exposed to increasing concentrations of the extract, initially there was no mortality in the pests but increase in the concentration led to paralyzing effects. Also, as the exposure time was increased, there was an increase in the mortality. Results indicated that *S. as per* exhibited significant contact toxicity against the stored grain pest *T. granarium* [37].

4.5 Antimicrobial

Studies were conducted to determine the antibacterial activity of the ethanolic extract as well as the acidic and basic fractions obtained from the ethanolic extract of the leaves of *S. asper* against *S. aureus* and *B. subtilis* (both gram positive) and *E. coli* and *P. aeruginosa* (both gram negative) using the broth microdilution method. The strongest antibacterial activity was exhibited by the acidic fraction against both the gram positive species. However the ethanolic extract as well as the fractions did not show any antibacterial activity against both the gram negative species [32].

Ethanolic extract of *S. asper* and several other plants used traditionally to treat the symptoms of tuberculosis in Laos were tested for their activity against three types of mycobacteria (virulent *Mycobacteriam tuberculosis* H37Rv (Mtb), non-replicating persistent Mtb (NRP Mtb) and *Mycobacterium smegmatis*). *S. as per* exhibited Minimum Inhibitory Concentration (MIC) of >100 µg/mL. Its IC_{50} against Verocells was also >100 µg/mL [39]. The results indicated that although it was traditionally used to alleviate the symptoms of tuberculosis, it was not found to be effective in the *in vitro* tests conducted.

4.6 Neuroprotective

Studies were carried out to investigate the neuroprotective and antiaging effects of the ethanolic extract of *S. as per* leaves as well as neutral, acidic and basic fractions obtained from it [32, 40-41].

Reduction in the oxidative toxicity induced by the excitatory neurotransmitter was glutamate used for assessing the ethanolic extract's neuroprotective effects in age related diseases. It was observed that the *S. asper* extract exhibited a reduction in the cytotoxicity caused by glutamate which was concentration dependent. The nematode *Caenorhabditis elegans* was used for evaluating the longevity effects of the extract. It was observed that it could extend the life span and prolong the survival of the nematode in its first larval stage although it failed to exhibit any effect on the late larval stages of the nematode suggesting that its longevity effects are best displayed when it is used for treatment at an early stage. Studies also suggested that the ethanolic extract of *S. as per* leaves protected the nematodes from aging and photoaging

via MAPK pathway and SKN-1. The studies thus led to the conclusion that it possessed neuroprotective and antiaging properties which also lent credence to its use in the Thai traditional formula for longevity [40-41].

In another set of experiments, studies were conducted to evaluate the neuroprotective activity of the ethanolic extract of the leaves of *S. asper* as well as the neutral, acidic and basic fractions obtained from it against glutamate-induced toxicity in hippocampal neuronal HT22 cells. It was observed that the crude extract as well as the neutral and basic fractions exhibited a dose dependent protective action against cytotoxicity induced by glutamate. The viability of the HT22 cells was significantly increased. However, the acidic fraction showed no protection. These extracts and fractions were also evaluated for their acetylcholine esterase inhibitory activity using the TLC-direct bioautography method, which showed the presence of AChE inhibitory compounds. The neutral fraction was found to exhibit the best neuroprotective activity [32].

Aqueous extract of the fresh leaves of *S. asper* was investigated for its effects on the level of the reactive oxygen species in H2O2-treated SK-N-SH cell cultures as well as its functional and behavioral effects in Parkinson's disease-like symptoms in male C57BL/6 mice. MPTP was used to induce the Parkinson disease like symptoms in the animals. SK-N-SH cell viability was measured by MTT assay after incubation with extract for 24 h. The ROS levels were found to be significantly decreased. The results also demonstrated that the extract could be used to reverse the functional changes in the MPTP treated mice, thereby indicating that it had potential anti-Parkinson disease activity [42].

4.7 Anticonvulsant

The stem bark of *S. asper* been reported to possess anticonvulsant activity in folk medicine. In order to scientifically validate these traditional claims, its n-hexane, dichloromethane and aqueous fractions were prepared, and they were subjected to different experiments for neuro-pharmacological disorders. The anticonvulsant activity was evaluated in mice using the maximal electroshock induced and the isoniazid induced convulsion models. The antidepressant activity was evaluated using the forced swim test and the tail suspension test in mice. Neurotoxicity studies were also conducted. The results indicated that n-hexane fraction was active in all the models tested for induced seizures [43].

4.8 Antitumor/ Antineoplastic/ Anticancer

Several workers have evaluated the stem bark of *S. asper* for its anticarcinogenic and antineoplastic activities [27, 44-46]. Different models as well as cell lines were used to study its antimitotic, cytotoxic and antitumor activities. Wheat seeds, either in their germinating stage or in their early seedling growth stage, were used as test material for determining the antimitotic activity of the extract.HT-29 human colon cancer cell line, using paclitaxel as the positive control as well as by brine shrimp nauplii lethality assay was used to evaluate its cytotoxicity. Its antitumor effects were determined by assessing inhibition of tumor growth on potato disc due to *Agrobacterium tumefaciens*, against Dalton's ascitic lymphoma (DAL) in Swiss albino mice and in high-grade serous ovarian cancer cell lines.

The ethanolic extract of the stem bark of *S. asper* was evaluated for its antimitotic activity using wheat seeds. Antimitotic activity or inhibition of cell division was measured by evaluating the seed germination counts as well as measuring the root lengths. Results indicated that there was inhibition of seed germination and elongation of roots indicating that the extract exerted antimitotic activity. The antimitotic activity was also found to be dose dependent with a higher concentration of the extract causing a greater inhibition and suppression of growth of the wheat seeds [44].

The brine shrimp nauplii lethality bioassay for cytotoxic activity was carried out using different concentrations of the ethanol extract of *S. asper* stem bark. The death rate of the nauplii was found to be directly related to the concentration of the extract and exposure time. The bark extract showed LC_{50} value of 45.21 µg/ml. The results indicate the presence of anticancer agents in the ethanolic extract of the stem bark of *S. asper* [44].

In yet another study, the anti-cervical cancer potential of *S. asper* was investigated through the identification of key proteins and their expression that are regulated in the treatment using mice xenograft model. These analyses may improve the molecular insight of the mechanisms involved in the treatment of cervical cancer tumour by *S. asper* extract [47].

The ethanol extract of stem bark of *S. asper*exhibited a dose dependent inhibitory effect in *Agrobacterium tumefaciens* induced tumors i.e. crown gall. Camptothecin was used as a positive control. The extract exhibited a significant inhibition of the growth of crown galls [44].

In a separate study, the stem bark of *S. asper* was extracted with methanol and its ethyl acetate fraction was tested for its antitumor activity. The tumor growth parameters were determined against Dalton's ascitic lymphoma (DAL) in Swiss albino mice on intraperitoneal administration of the fraction at two different dose levels. Its antioxidant properties were also studied. The fraction was found to decrease the growth of the tumor in a dose dependent manner as well as increased the rate of survival of the animals. The overall results indicated that the stem bark of *S. asper* possessed good antitumor activity [45].

The stem bark of *S. asper* was extracted with chloroform. This extract was found to exhibit cytotoxic activity when tested against HT-29 human colon cancer cell lines. Its bioassay guided fractionation yielded several cardiac glycosides, including strebloside. All compounds, along with a commercially available sample of digoxin, were tested for their cytotoxicity toward the HT-29 cell line. All compounds tested showed potent cytotoxicity that was comparable with digoxin, with IC_{50} values in the range 93–690 nM, indicating that they were the cytotoxic principles of the stem bark of *S. asper*. The major active compound, (+)-strebloside, and digoxin were also tested for their selectivity, using normal human CCD-112CoN colon, NL20 lung, and peripheral blood mononuclear cells. The potency of both agents toward normal human cells was found to be much lower than that against human cancer cells, indicating that both strebloside and digoxin showed selective cytotoxicity toward human colon and lung cancer and leukemia cells. The selectivity of strebloside observed toward HT-29 cells and CCD-112CoN cells was greater than digoxin indicating it to possess potential antineoplastic activity (Ren *et al*., 2017). Molecular docking studies and *in vitro* assay showed that (+) strebloside behaved in a manner similar to digitoxin where its binding to and inhibiting Na+/K+ ATPase was concerned. Use of strebloside inhibited the growth of several high-grade ovarian cancer cells. Other studies also confirmed its antitumor potential. However, it was highly similar to that of other more

well-known cardiac glycosides in its overall biological activity, and therefore this compound could likely suffer from the same side effects [46].

In another study, the cytotoxic chloroform fraction of the methanolic extract of the aerial parts of *S. asper* comprising of the flowers, leaves, and twigs, yielded (+)-17β-hydroxystrebloside, (+)-3′-de-O-methylkamaloside and (+)-strebloside when it was subjected to bioactivity guided separation. Inhibitory activity against the HT-29 human colon cancer cell line was used. Of the three compounds isolated, (+)-17β-hydroxystrebloside was a new but non-cytotoxic cardiac glycoside whereas the other two were known cytotoxic compounds [48].

4.9 Analgesic

The ethanolic extracts of the leaves and bark of *S. asper* were investigated for their analgesic properties. Acetic acid induced writhing test was performed to assess the analgesic effects of the extracts at two different doses of 500 and 250 mg/kg body weight. Significant inhibition of writhing was observed. The leaf extract produced 65.46 and 27.79% inhibition of writhing while the bark extract produced 58.70 and 20.26% inhibition of writhing respectively [3].

4.10 Orodental

Previous studies have reported that *S. asper* leaf extract (SAE) possessed antibacterial activity towards caries associated bacteria, endodontic and interferes with the *in vitro* adherence of Candida to human buccal epithelial cells and acrylic surface [5].

It has been observed that SAE interferes with the *in vitro* adherence of *Candida* to human buccal epithelial cells and acrylic surface and that 60% of the seniors using a full denture suffer from denture stomatitis with *Candida albicans* being the main causative agent. The inhibitory effects of various sublethal concentrations of (SAE) on the *in vitro* adhesion of *C. albicans* to denture acrylic were therefore also studied. The results indicated that the extract reduced the adhesion capability of *Candida* cells to the denture acrylic, thereby reducing the chances of denture stomatitis in denture users [49].

In vitro experiments were carried out using the SAE to study its inhibitory effects on the formation of subgingival biofilms. A count of the *P. gingivalis, A. actinomycetemcomitans* and total bacteria was determined in the subgingival biofilms cultivated from the subgingival plaque samples of periodontitis patients along with different concentrations of the extract and compared with the untreated control. The results indicated that the extract inhibited the *in vitro* formation of the biofilm and also reduced the count of the *P. gingivalis, A. actinomycetemcomitans* and total bacteria [50].

In another study, antimicrobial effect of mouthrinse containing S. asper extract on salivary *S. mutans* and *A. actinomycetecomitans* was compared with that of a chemical mouthwash containing chlorohexidine and it was concluded that SAE exhibited a significant effect on gingival health without significant effect on plaque growth. Thus, an additional usage of SAE mouthrinse to routine mouth cleaning may enhance the protective value to oral hygiene [51].

4.11 Safety toxicity studies

The methanol and petroleum f. In acute toxicity studies both the extracts were found to be safe up to the dose of 2,000 mg/kg., b.w. given orally. In the sub-chronic safety studies, the methanolic extract was found to be weakly toxic whereas the petroleum ether extract was found to be non-toxic [52].

Acute toxicity studies of the petroleum ether extract of the stem bark of *S. asper* showed that it was non-toxic. The test animals were kept under observation for 2 h to check their behavioral, neurological and autonomic profiles and were under observation for 72 h to see the effect of the extract on their mortality and toxicity. The extract was found to be safe and non-toxic up to a dose of 1500 mg/kg, b.w. administrated orally [35].

Conclusions

Streblus asper has traditionally been used as medicine in different parts of the world especially in the South Asian countries like India, Bangladesh, Malaysia, Thailand and Pakistan as well as in China. Previous studies have shown it to be a rich source of cardiac glycosides with as many as 20 cardiac glycosides being isolated from the roots of this plant. Several cardiac glycosides that were recently isolated along with strebloside, exhibited potential anticancer activity. However, amongst these, strebloside was found to be the most promising one. The selectivity of strebloside observed toward HT-29 cells and CCD-112CoN cells was greater than digoxin indicating it to possess potential antineoplastic activity. It is well known that cardiac glycosides are highly toxic. Digoxin and ouabain, which are cardiac glycosides, are often used for the treatment of congestive heart failure, but at a very low dose. Therefore, a very cautious approach is required in exploiting and developing this group of compounds for any therapeutic and pharmaceutical use. These compounds need to be structurally modified or altered in such a way that they exhibit lesser adverse effects yet retain their anticancer potential.

During the past few years, several lignans and neolignans, another group of biologically active compounds, have been isolated and identified from this plant. These are cinnamic acid or phenylpropanoid derivatives formed as a result of oxidative coupling. Podophyllotoxin, etoposide, teniposide, terminalos ide P, schibitubin B, patentiflorin A and schisanwilsonin G are some well-known biologically active compounds belonging to this group known to exhibit antileukemic, antitumor, anti-inflammatory, neuroprotective, anti-neurodegenarative and antiviral activities[53,54]. *S. asper* has yielded several biologically active lignans and neolignans specially exhibiting antiviral activity against hepatitis B virus. *viz*. (7’R,8’S,7’’R,8’’S)-erythro-strebluslignanol G (18), (7’S,8’S)-trans-streblusol A (6), (7’R,8’S)-erythro-streblusol B (7), (7’S,8’S)-threo-streblusol B (8), 8’R-streblusol C (9) and (8R,8’R)-streblusol D (10). Amongst these, (7’R,8’S,7’’R,8’’S)-erythro-strebluslignanol G (18) exhibited significant anti-hepatitis B virus activity with an IC50 of 1.6 mM. These compounds can be used as lead compounds or as chemical scaffolds for the development of anti-hepatitis B virus agents.

Present as well as previous studies carried out on *S. asper* (known as ‘toothbrush tree’) leaf extracts have shown that it exhibited antibacterial activity towards caries associated bacteria, endodontic pathogens and interfered with the *in vitro* adherence of Candida to human buccal epithelial cells. It inhibited the formation of subgingival biofilms as well as reduced the adhesion capability of Candida cells to the denture acrylic, thereby reducing the chances of denture stomatitis in denture users. These advantages can be exploited for developing products for use in orodental care. Besides, the studies conducted on the factors that affect the milk

coagulating properties of its leaf extract [55] would be useful in utilizing the protease present in the leaves of *S. asper* as a potential substitute for rennet which is the enzyme commonly used in the cheesemaking industry.

Detailed pharmacological investigations of *S. asper* have shown it to exhibit anti-inflammatory, antioxidant, antimicrobial, antibiofilm and anticancer activities as well as given credence to its use for the treatment of various diseases such as dysentery, relief of toothache, antigingivitis, filariasis, epilepsy, epistaxis, piles and stomachache. They provide a plausible evidence for its multifarious therapeutic uses in the traditional systems of medicine. However, well organized, systematic, and in-depth studies are needed, especially safety studies, in order to exploit the full potential of this medicinal plant.

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Conflict of interest

The authors have no conflict of interest to declare.

References

1. Anonymous. The Ayurvedic Pharmacopoeia of India, Vol. III, Part I. Department of ISM and Homoeopathy, Ministry of Health and Family Welfare, Delhi, 2001, 460.
2. Gupta AK, Tandon N, Sharma M. Quality Standards of Indian Medicinal Plants, New Delhi: Indian Council of Medical Research. 2005;2:227-34.
3. Afjalus SM, Salahuddin M, Rahman M, Khatun A, Yasmin F. Investigation of analgesic and antioxidant activity of ethanolic extract of the leaf and bark of Streblus as per Lour. Int. Res. J. Pharm. 2013;4:262-266.
4. Rastogi S, Kulshreshtha DK, Rawat AKS. Streblus asperLour. (Shakhotaka): A Review of its Chemical, Pharmacological and Ethnomedicinal Properties. Evid. Based Complement. Alternat. Med. 2006;3(2):217-222.
5. Taweechaisupapong S. Role of Streblus asper in Systemic and Oral Health: An Overview. J. Dent. Assoc. Thai. 2015;65:60-66.
6. Prakash S. A Review on Chemical and Pharmacological Profiles of Shakhotaka (Streblus asper Lour.). Anveshana Ayur. Med. J. 2015;1:256-262.
7. Kadir MF. Sayeed MSB, Setu NI, Mostafa A, Mia MMK. Ethnopharmacological survey of medicinal plants used by traditional health practitioners in Thanchi, Bandarban Hill Tracts, Bangladesh. J. Ethnopharmacol. 2014;155:495-508.
8. Zheng X, Wei J, Sun W, Li R, Dai H. Ethnobotanical study on medicinal plants around Limu Mountains of Hainan Island, China. J. Ethnopharmacol. 2013;148(3):964-974. DOI: 10.1016/j.jep.2013.05.051.
9. Saikia AP, Ryakala VK, Sharma P, Goswami P, Bora U. Ethnobotany of medicinal plants used by Assamese people for various skin ailments and cosmetics. J. Ethnopharmacol. 2006;106:149-157.
10. Choudhury PR, Choudhury MD, Ningthoujam SS, Mitra A, Talukdar AD. Plant utilization against digestive system disorder in Southern Assam, India. J. Ethnopharmacol. 2015;175:192-197.
11. Tarafdar RG, Nath S, Talukdar AD, Choudhury MD. Antidiabetic plants used among the ethnic communities of Unakoti district of Tripura, India. J. Ethnopharmacol. 2015;160:219-226.
12. Shil S, Choudhury MD, Das S. Indigenous knowledge of medicinal plants used by the Reang tribe of Tripura state of India. J. Ethnopharmacol. 2014;152:135-141.
13. Panda SK. Ethno-medicinal uses and screening of plants for antibacterial activity from Similipal Biosphere Reserve, Odisha, India. J. Ethnopharmacol. 2014;151:158-175.
14. Atjanasuppat K, Wongkham W, Meepowpan P, Kittakoop P, Sobhon P, Bartlett A, *et al*. *In vitro* screening for anthelmintic and antitumour activity of ethnomedicinal plants from Thailand. J. Ethnopharmacol. 2009;123:475-482.
15. Modak BK, Gorai P, Pandey DK, Dey A, Malik T. An evidence based efficacy and safety assessment of the ethnobiologicals against poisonous and non-poisonous bites used by the tribals of three westernmost districts of West Bengal, India: Anti-phospholipase A2 and genotoxic effects. PLoS ONE. 2020;15(11):e0242944. https://doi. org/10.1371/journal.pone.0242944
16. Sahoo AK, Behera HC, Behura AK. Ethnomedicine and Traditional Health Care System of a Particular Vulnerable Tribal Group in India: Application of Plant Extracts. Res. Square. 2020. DOI: https://doi.org/10.21203/rs.3.rs-25202/v1
17. Swargiary A, Daimari M, Roy MK. Survey and documentation of anthelmintic plants used in traditional medicine system of tribal communities of Udalguri district of Assam, India. J. of App. Pharm. Sci. 2020;10(1):046-054.
18. Aziz MA, Adnan M, Khan AH, Sufyan M, Shahid Niaz Khan SN. Cross-cultural analysis of medicinal plants commonly used in ethnoveterinary practices at South Waziristan Agency and Bajaur Agency, Federally Administrated Tribal Areas (FATA), Pakistan. J. Ethnopharmacol. 2018;210:443-468. doi: 10.1016/j.jep.2017.09.007.
19. Neamsuvan O, Tuwaemaengae T, Bensulong F, Asae A, KholeelMosamae K. A survey of folk remedies for gastrointestinal tract diseases from Thailand's three southern border provinces. J. Ethnopharmacol. 2012;144:11-21.
20. Li J, Zhang Y, Jin B, Su X, Tao Y, Shea Z, Lin Y. 1H and 13C NMR assignments for two lignans from the heartwood of Streblus asper. Magn. Reson. Chem. 2008;46(5):497-500. doi: 10.1002/mrc.2186.
21. Li L, Li J, Huang Y, Wu Q, Deng S, Su X, Yang R, *et al*. Lignans from the heartwood of Streblus asper and their inhibiting activities to Hepatitis B virus. Fitoterapia. 2012;83(2):303-309. doi: 10.1016/j.fitote.2011.11.008.
22. Chen H, Li J, Wu Q, Niu X, Su X. Anti-HBV activities of Streblus asper and constituents of its roots. Fitoterapia. 2012;83(4):643-649.doi: 10.1016/j.fitote.2012.01.009.
23. Li J, Huang Y, Guan X, Li J, Deng S, Wu Q, *et al*. Anti-hepatitis B Virus constituents from the stem bark of Streblus asper. Phytochem. 2012;82:100-109. doi: 10.1016/j.phytochem.2012.06.023.
24. Li J, Meng A, Guan X, Li J, Yang R. Anti-hepatitis B virus lignans from the root of Streblus asper. Bioorg. Med. Chem. Lett. 2013;23(7):2238-2244. doi: 10.1016/j.bmcl.2013.01.046.

25. Nie H, Guan X, Li J, Zhang Y, Li J. Antimicrobial lignans derived from the roots of Streblus asper. Phytochem. Lett. 2016;18:226-231.
26. Aeri V, Alam P, Ali M, Naquvi KJ. Lupene-type triterpenic and steroidal constituents from the roots of Streblus asperLour. J. Sci. Innov. Res. 2015;4:142-145.
27. Ren Y, Chen WL, Lantvit DD, Sass EJ, Shriwas P, Ninh TN, *et al*. Cardiac glycoside constituents of Streblus asper with potential antineoplastic activity. J. Nat. Prod. 2017;80(3):648-658. doi: 10.1021/acs.jnatprod.6b00924.
28. Tripathi P, Tomar R, Jagannadham MV. Purification and biochemical characterisation of a novel protease streblin. Food Chem. 2011;125(3):1005-1012. DOI: 10.1016/j.foodchem.2010.09.108.
29. Kumar RBS, Kar B, Dolai N, Bala A, Haldar PK. Antioxidant and hypoglycemic property of Streblus asper in streptozotocin induced diabetic rats. Asian Pac. J. Trop. Dis. 2012;2:139-143.
30. Gadidasu K, Reddy ARN, Umate P, Reddy YN, Abbagani S. Antioxidant and anti-diabetic activities from leaf extracts of Streblus asperLour. BioTech. Indian J. 2009;3:231-235.
31. Ibrahim NM, Mat I, Lim V, Ahmad R.. Antioxidant activity and phenolic content of Streblus asper leaves from various drying methods. Antioxidants (Basel). 2013;2(3):156-66. doi: 10.3390/antiox2030156.
32. Prasansuklab A, Theerasri A, Payne M, Ung AT, Tencomnao T. Acid-base fractions separated from Streblus asper leaf ethanolic extract exhibited antibacterial, antioxidant, antiacetylcholinesterase, and neuroprotective activities. BMC Complement. Altern. Med. 2018;18(1):223. doi: 10.1186/s12906-018-2288-4.
33. Kumar RBS, Dolai N, Karmakar I, Bhattacharya S, Haldar PK. Evaluation of *in vitro* antioxidant activity of Streblus asper Bark. Global J. Pharmacol. 2016;10:1-5.
34. Karan SK, Mishra SK, Pal DK, Singh RK, Raj G. Antidiabetic effect of the roots of Streblus asper in alloxan-induced Diabetes mellitus. Asian J. Chem. 2012;24"422-424.
35. Karan SK, Mondal A, Mishra SK, Pal DK, Rout KK. Antidiabetic effect of Streblus as per in streptozotocin-induced diabetic rats. Pharm. Biol. 2013;51(3):369-75. doi: 10.3109/13880209.2012.730531
36. Rahman MO, Alqahtani AS, Huda SB, Siddiqui SA, Noman OM, Nasr F, *et al*. Streblus asper attenuates alloxan-induced diabetes in rats and demonstrates antioxidant and cytotoxic effects. Pharm. Biol. 2021;59(1):1058-64.
37. Nasrin F, Mahrin N, Jahan N, Begum Y, Majumder S. *In vitro* membrane stabilizing and insecticidal activities of methanolic extract of Streblus as per Lour. Pharma Tutor. 2015;3:29-34.
38. Sripanidkulchai B, Junlatat J, Wara-aswapati N, Hormdee D. Anti-inflammatory effect of Streblus asper leaf extract in rats and its modulation on inflammation-associated genes expression in RAW264.7 macrophage cells. J Ethnopharmacol. 2009;124(3):566-570. doi: 10.1016/j.jep.2009.04.061.
39. Elkington BG, Sydara K, Newsome A, Hwang CH, LAN KIN DC, Simmler C, *et al*. New finding of an anti-TB compound in the genus Marsypopetalum (Annonaceae) from a traditional herbal remedy of Laos. J. Ethnopharmacol. 2014;151:903-911.
40. Prasansuklab A, Meemon K, Sobhon P, Tencomnao T. Ethanolic extract of Streblus asper leaves protects against glutamate-induced toxicity in HT22 hippocampal neuronal cells and extends lifespan of Caenorhabditis elegans. BMC Complement. Altern. Med. 2017;17(1):551. doi: 10.1186/s12906-017-2050-3
41. Prasanth MI, Brimson JM, Malara DS, Prasansuklab A, Tencomnao T. Streblus asper Lour. exerts MAPK and SKN-1 mediated anti-aging, anti-photoaging activities and imparts neuroprotection by ameliorating A in Caenorhabditis elegans. Nutrition and Healthy Aging. 2021. DOI 10.3233/NHA-210121
42. Singsai K, Akaravichien T, Kukongviriyapan V, Sattayasai J. Protective Effects of Streblus asper Leaf extract on H_2O_2-Induced ROS in SK-N-SH cells and MPTP-induced Parkinson's disease-like symptoms in C57BL/6 mouse. Evid. Based Complement. Alternat. Med. 2015, 970354. doi: 10.1155/2015/970354.
43. Verma V, Tripathi AC, Saraf SK. Bioactive non-sterol triterpenoid from Streblus as per: microwave-assisted extraction, HPTLC profiling, computational studies and neuropharmacological evaluation in BALB/c mice. Pharm Biol. 2016;54(11):2454-2464.
44. Alamgir A, Rahman M, Rahman A. Phytochemical characteristics, antimitotic, cytotoxic and antitumor activities of bark extract of Streblus asperLour. Bangladesh J. Bot. 2013;42:17-22.
45. Kumar RB, Kar B, Dolai N, Karmakar I, Bhattacharya S, Haldar PK. Antitumor activity and antioxidant status of Streblus asper bark against Dalton's ascitic lymphoma in mice. Interdiscip. Toxicol. 2015;8(3):125-30. doi: 10.1515/intox-2015-0019.
46. Chen WL, Ren Y, Ren J, Erxleben C, Johnson ME, Gentile S, *et al*. (+)-Strebloside-induced cytotoxicity in ovarian cancer cells is mediated through cardiac glycoside signaling networks. J Nat Prod. 2017;80(3):659-669. doi: 10.1021/acs.jnatprod.6b01150.
47. Nabil M, Seeni A, Ismail WI, Mail MH, Rahim NA. Changes in the Protein Profile of Cervical Cancer Mice Xenograft Model in Response to Streblus asper Treatment. Journal of Natural Remedies, 2020 July, 20(3).
48. Ren Y, Tana Q, Heath K, Sijin Wua S, *et al*. Cytotoxic and non-cytotoxic cardiac glycosides isolated from the combined flowers, leaves, and twigs of Streblus as per. Bioorg Med Chem. 2020;28(4):115301. doi:10.1016/j.bmc.2019.115301.
49. Taweechaisupapong S, Klanrit P, Singhara S, Pitiphat W, Wongkham S. Inhibitory effect of Streblus asper leaf-extract on adhesion of Candida albicans to denture acrylic. J. Ethnopharmacol. 2006;106:414-7.
50. Taweechaisupapong S, Pinsuwan W, Suwannarong W, Kukhetpitakwong R, Luengpailin S. Effects of Streblus asper leaf extract on the biofilm formation of subgingival pathogens. S. Afr. J. Bot. 2014;94:1-5S.
51. Kumar G, Kanungo S, Panigrahi K. Antimicrobial effects of Streblus asper leaf extract: A randomized controlled clinical trial. J of Pharmacol & Clin Res. 2020;8(3):555740. DOI:10.19080/JPCR.2020.08.555740
52. Kumar RBS, Puratchikodi A, Prasanna A, Narayan Dolai N, Majumder P, Mazumder UK, *et al*., Preclinical studies of Streblus asperLour in terms of behavioural safety and toxicity. Orient. Pharm. Exp. Med. 2011;11:243-249.
53. Teponno RB, SouvikKusari S, Spiteller M. Recent advances in research on lignans and neolignans. Nat.

Prod. Rep. 2016;33(9):1044-1092. doi: 10.1039/c6np00021e.
54. Zálešák F, Bon DJD, Pospíšil J. Lignans and Neolignans: Plant secondary metabolites as a reservoir of biologically active substances. Pharmacol Res. 2019;146:104284. doi: 10.1016/j.phrs.2019.104284.
55. Ishak R, Ahmed Y, Mustafa S, Sipat A, Muhammad K, Abd Manap Y. Factors affecting milk coagulating activities of Kesinai (Streblus asper) Extract. Int. J. Dairy Sci. 2006;1:131-135.