Research Article

Evaluation of Anxiolytic Potential of *Streblus asper* Lour in Experimental Animal Models

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ABSTRACT

Objective: The main goal of the current study is to examine the anxiolytic potential of methanolic extract of *Streblus asper* Lour leaves on experimental animal models.

Methods: The Forced Swim Test, Tail Suspension Test, Dark and Light Exploratory Test, Open Field Test, and Staircase Experimental Models are chosen for evaluating the anxiolytic activity of test substance. For this study 25–30 grams of adult Swiss albino mice were chosen and divided into four groups, five animals in each group among the various experimental animal models. Before that acute oral toxicity studies were performed and lower (200 mg/kg) and higher (400 mg/kg) test doses of methanolic extract of *Steblus asper* lour were selected for present study. The divided four groups are treated with control (normal saline 15ml/kg), standard (Diazepam 4 mg/kg), test dose -1(200 mg/kg) and test dose-2 (400 mg/kg) respectively in all experimental models. All the findings were analyzed using a one-way analysis of variance (ANOVA). P was deemed to have statistical significance.

Result: The test drugs have shown significant results in each model when compared to the standard Diazepam. The observations of each model are like an increase in immobility time was seen in both forced swimming test and tail suspension test, there is a decrease in number of squares crossed and rearings in open field test, time spent in light chamber was increased compared to dark chamber in case of dark and light chamber model and number of step climbing's are decreased in staircase method. When compared all the treatment groups with control the results are statistically significant ($p<0.0001$****). Compared to lower dose of test and standard Diazepam, the higher dose of test drug has shown appreciable anxiolytic potential.

Conclusion: According to the current study's finding, methanolic leaf extract of *Streblus asper* Lour has good anxiolytic potential. We can suggest our product for further research and development purposes for further molecular level of studies.

Keywords: Anxiety, *Streblus asper* Lour, Diazepam, Swiss albino mice, anti-anxiety models.

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INTRODUCTION

The complexity of daily life in modern civilization regularly causes varied degrees of distress.[1] It can be described as a subjective feeling of dread, foreboding, or unease that is accompanied by an expectation of approaching danger. It can also be a sign of a primary psychological disorder or a primary medical issue.[2] Ten to twenty million suicide attempts occur each year in the world and many of them are brought on by mental illnesses. Sadly, there is a lack of awareness and treatment for many conditions.[3]

Although anxiety is a common emotional response, when it is extreme, it can develop into a pathological condition that can lead to heart disease and other psychological and physical problems.[4] An issue with the central nervous system's ability to control it can cause anxiety.[5] In addition, they may experience a mood condition that comes on suddenly and for no apparent reason. It differs from terror because it doesn't occur when a threat is sensed. Additionally, fear is associated with complex responses like running away and avoiding situations, whereas anxiety results from hazards that are perceived to be uncontrollable or immediate.[6]

Stress is the primary cause of anxiety, although hereditary characteristics like anxiety features also increase the likelihood of experiencing anxiety.[7] These conditions are regulated by inhibitory and facilitative mechanisms that either mitigate or enhance anxiety states.[8]

Serotonin-norepinephrine reuptake inhibitors, including venlafaxine and duloxetine, are regarded as useful medications. However, they do have certain negative effects, such as raising systemic blood pressure.[5] The main class of drugs used in treating anxiety are benzodiazepines, and this class of drugs continues to be the most widely prescribed one.[4] Despite the fact that benzodiazepines' clinical applications are constrained by side effects such psychomotor impairment, potentiation of other central

depressants, and dependence risk, benzodiazepines are nonetheless used in some situations.[1]

The limitations of current drug therapies for anxiety include their high cost, their slow onset, their lack of effectiveness, their unwanted side effects, their dependence, and the stigma attached to using and relying on pharmaceuticals.[9]

Indigenous medicines are currently being used to treat a variety of illnesses, and herbal remedies are seeing a worldwide resurgence. As of right now, synthetic products are viewed as dangerous to both humans and the environment due to their negative effects, whereas herbal remedies are currently deemed to be safe. People are turning away from synthetic items in favor of natural ones these days since they are more dependable and safer.[3] Herbal medicine, often known as herbalism or botanical medicine, is a type of treatment that relies on the consumption or topical application of plant materials.[10]

Small trees like *Streblus asper* Lour, a member of the *Moraceae* family, are native to tropical regions including the Philippines, Malaysia, Sri Lanka, and India.[11] Elephantiasis, leprosy, piles, and tuberculosis are just a few of the illnesses that are traditionally treated with it. This plant produces a milky liquid that is both astringent and antibacterial. Diarrhea, dysentery, and fever can all be treated with a decoction of the bark. As a remedy for snake poison as well as for sinusitis and ulcers, the roots are employed. Twigs are utilized for pyorrhoea, while the plant's latex is used to treat elephantiasis and glandular swellings.[12] In addition, *Streblus asper* Lour leaf extracts have recently been revealed to possess Neuroprotective qualities in both *in-vitro* and *in-vivo* models of neurodegenerative disorders.[13]

This main goal of the present investigation was to provide current, thorough information on the anti-anxiety effects of methanolic leaf extracts of *Streblus asper* Lour.

MATERIALS AND METHODS

Collection of plant material and authentication

The leaves of *Streblus asper* Lour belonging to the family *Moraceae* were collected on July 08, 2022 from the local areas of the Yermal Bada, Udupi district Karnataka, India. The plant material was identified and authenticated by botanist Dr H.S. Shenoy, M.Sc., M.Phil., Ph.D., Principal Scientist and Head of Botany Division, and the voucher specimen (voucher number=2637) was deposited in the Pilikula Herbarium Recognized by index Herbarium of New York Botanical Garden (NYBG)

Processing of test sample

The leaves of *Streblus asper* Lour plant were washed with tap water to remove impurities and dried under the shade at room temperature for about one month and then powdered using miller. This powder sample was kept in an airtight container and used for subsequent extractions.

Methanolic Extraction of *Streblus asper* Lour leaves

Simple maceration procedure was chosen for extraction process. The powdered leaves of *Streblus asper* Lour were further reduced to coarse powder by using sieve number 40 size and was used for the extraction process. The 100 grams of finely powdered leaf sample was macerated with 500ml of Methanol in an Erlenmeyer flask for 7 days at room temperature. The extraction process was facilitated by occasional shaking. Then, the mixture was first filtered by passing it through a muslin cloth and later on with Whatman filter paper, #1. The filtrate of methanolic leaf extract of *Streblus asper* Lour was collected and evaporated to dryness to give a final crude extract. The yield of dark-green semi solid extract was stored at 4°C in an amber-colored bottle until required for experiments. Then extracted material was submitted to phytochemical analysis.

Drugs and Chemicals

Diazepam is a potent anxiolytic agent used as a standard drug, and all other chemicals and reagents like Tween 80, Distilled water, Methanol, Normal saline were used of analytical grade.

Qualitative analysis of active constituents

1g of *Streblus asper* Lour was dissolved in 10.0 ml of methanol, left overnight, and filtered. Using a Linomat 5 TLC applicator, 4, 8 and 12 l of each of the aforementioned extracts were applied to a pre-coated silica gel F254 on aluminum plates with a band width of 7 mm. The plate was developed in ethyl acetate: methanol (9.6: 0.4). The produced plates were seen in short UV, long UV, and scanned under UV 254 nm and 366 nm. Rf, the color of the spots, and a densitometric scan were noted.

Experimental animals

Anxiolytic activity was studied in healthy adult male Swiss albino mice weighing between 25 and 30 grams. Animals were obtained from the institutional animal house facility and randomly divided into four groups of five in each. They were housed in polypropylene cages with husk bedding and fed standard pellet diet and water indefinitely. The animals were kept at a temperature of 25±2°C and on a 12-hour light and dark cycle. Animal experiments were conducted with the proper approvals of the Institutional Animal Ethics Committee (IAEC) and in strict accordance with its guidelines.

Acute oral toxicity study

According to OECD guidelines, the LD50 was determined in mice. To calculate the LD50 value in experimental animals, acute toxicity was typically used.[7] The mice were fasted for 3-4 hours before the experiment. The mice were given the medication utilizing oral gavage.[14] The methanolic extract of *Streblus asper* Lour was administered orally at dose of 300,500,1000,2000,3000 mg/kg body weight.[15] Mortality (if any) and or morbidity were observed for any toxicity symptoms continuously for 1 hour, sporadically for 4

hours, throughout the course of 24 hours, and continued for 14 days.[14] The dose given was thought to be hazardous if any mortality or severe morbidity have observed in two out of three animals. The same amount was administered again to confirm the harmful effect if only one animal out of three died. If no morbidity or mortality, then only the higher doses are employed in further studies.[15]

Experimental design:

The experimental animals were randomly grouped into four of five animals in each. Group I was treated with vehicle (normal saline 15ml/kg given orally) and considered as control, Groups II treated with diazepam (4 mg/kg given orally) and considered as standard, and Group III & IV treated with methanolic extract of *Streblus asper* Lour of 200 and 400 mg/kg respectively, and these two groups were considered as test -1 and test-2. The above experimental design was used similarly in all experimental models.

Experimental models for anti-anxiety activity:

Tail suspension test (TST): Stern et al. described the most widely used behavioral model for screening anxiety and depression-related activities in mice. Prior to the experiment, the animals were moved from the institutional animal house to the laboratory. They were placed in cages and given 1-2 hours to acclimate to laboratory conditions. Each mouse was suspended individually from the edge of the table 50 cm from the floor using tape 1 cm from the tip of the tail. During testing, each test animal was audibly and visually separated from the other animals. For 6 minutes, the total duration of immobility was manually recorded. Immobile animals were those who had no body movement, were passively suspended, or were completely motionless. The test was conducted in a dimly lit room, and each mouse was used only once during the experiment.[16]

Dark and Light exploratory test: The light -dark box test is a popular animal model used to assay unconditional anxiety responses in rodents. This apparatus is made up of wood and consists of two chambers. The dark chamber is 1/3 of the total box area and the size of this chamber is approximately 20 x 30 x 35 cm, which was painted black color and illuminated with a dim red light and closed on the top. The light chamber consists of 2/3 of the total area of the apparatus and its size is approximately 30 x 30 x 35 cm, which was painted with white color and brightly illuminated with a 100w white light source. The two rooms were connected by a small open door with a size of 7.5 x 5 cm at floor level in the middle of the partition. Each mouse was individually placed in the center of the light compartment and over the next 5 minutes observed for the number of times it passed through the two compartments and the time spent in the light and dark compartments. A diazepam dose of 4 mg/kg orally was used as the reference standard.[17]

Open field test: The open-field test is used to measure exploratory and locomotor activity in rodents. To prevent escape, the apparatus consists of an arena surrounded by high walls, and the open field's floor is divided into squares. The number of square crossings, rearing, and time spent moving are used in the test session to assess the rodent's activity. The open-field test can easily measure manic-like behaviors in rats and mice, such as hyperactivity, risk-taking behavior, and increased stereotypy. Crossing and rearing behaviors are used to assess hyperactivity in the open-field apparatus. Crossings refer to the total number of square crossings performed during the test period to measure the animals' locomotor activity. Rearings are the total number of erect postures adopted by the rodent with the intention of exploring during the test period. In general, the open field test may cause anxiety in animals due to social isolation and fear of novel environments and open spaces. Several studies have linked social isolation to stress-related indicators. The apparatus consists of an expanded court of a square arena (60 x 60 cm), with a white floor divided into 36 squares (10 x 10 cm), surrounded by continuous 25 cm high walls of Black Plexiglas. The arena is illuminated by two red lights (2 x 60 W) placed in the center. In this test, the 20 squares adjacent to the wall represent a protected field, known as the "periphery of the arena", while the other 16 squares represent an exposed field, or "center of the arena".

The experiment began with a single mouse placed in the center of the arena and allowed to roam freely for 5 minutes.

The animals were given a corresponding treatment and then placed individually in the square of the open field 30 minutes later.

For 5 minutes, the following parameters were monitored.

1. Spontaneous ambulation (number of squares crossed out at periphery)

2. Rearing (Number of times the animal stands on its hind legs).[18]

Staircase Method: The stairs consist of five identical steps. 2.5 cm in height and 10 cm in depth. The internal height of the walls remains constant throughout the stairwell. Each animal can be used only once. The mice were placed individually on the floor of the box, back up the stairs, at the end of the experiment. Over a 3-minute period, the total number of steps climbed and total number of readings are recorded. Only when the rat has four feet on the step is it considered climbed.[19]

Forced swim test (FST): Porsolt et al. describe the forced swim test, the most commonly used behavioral model for screening antianxiety-like activity in rodents. The procedure remained the same. Individual mice were swum in an open glass chamber (25 x 15 x 25 cm) containing fresh water to a depth of 15 cm and kept at 26°±1 °C. Animals were unable to support themselves at this water level by touching the chamber's floor or side walls with their hind

legs or tail. Because it has been shown that spending in water can alter the behavior in rodents, the water in the chamber was changed after each animal received the test. During the first two minutes of testing, each animal moved quickly. The duration of immobility was manually recorded over the next 4 minutes of the total 6-minute test period. Mice were considered immobile if they stopped fighting and floated motionless in water, making only the movements required to keep their heads above the surface. Mice were towel dried and returned to their home environment after swimming.[16]

Statistical analysis: Results obtained were analyzed by using one-way ANOVA method followed by Dunnett's test. All the results were expressed as the mean ± standard error of the mean (SEM). All the treatment groups were compared with control. P values less than 0.005 was considered as statistically significant.

RESULTS

Qualitative analysis

Streblus asper Lour leaf extract in methanol was subjected to densitometric analysis, Rf values, and HPTLC picture documentation. Strebloside, Asperoside, and other compounds have Rf values of 0.48 0.02, 0.66 0.04, and 0.84 respectively. Rf values of methanolic leaf extract of *streblus asper* lour have been given in table 1. HPTLC reports of methanolic leaf extract of *streblus asper* lour have been depicted in figure 1. Densitometric scans of Methanolic leaf extract of *Streblus asper* have been depicted in figure 2.

Acute oral toxicity study

Acute oral toxicity studies done by up-down regulation method. Five male albino mice were administered an initial dose by oral route of 300, 500, 1000, 2000, and 3000 mg/kg body weight, with one being kept normal. There were no observations related to morbidity and mortality in the case of mice treated with several doses of methanolic leaf extract from *Streblus asper* Lour at 300, 500, 1000, 2000, and 3000 mg/kg. Because their gross and net weights were not significantly different when compared with control values.

Tail suspension test [TST]

The tail suspension test measures the total amount of immobility that a mouse experiences while having its tail suspended. Animals treated with standard drug and two doses of *Streblus asper* Lour leaf extract (200 and 400 mg/kg), became more immobile in the tail suspension test. This increase in immobility was significant with standard, low and high doses of test drug when compared to a control group that received only normal saline. These results were statistically significant with p<0.0001****, indicating a comparable anxiolytic effect of the test drug when compared to standard. diazepam (4 mg/kg). Observations on immobility time in tail suspension model have been given in table 2.

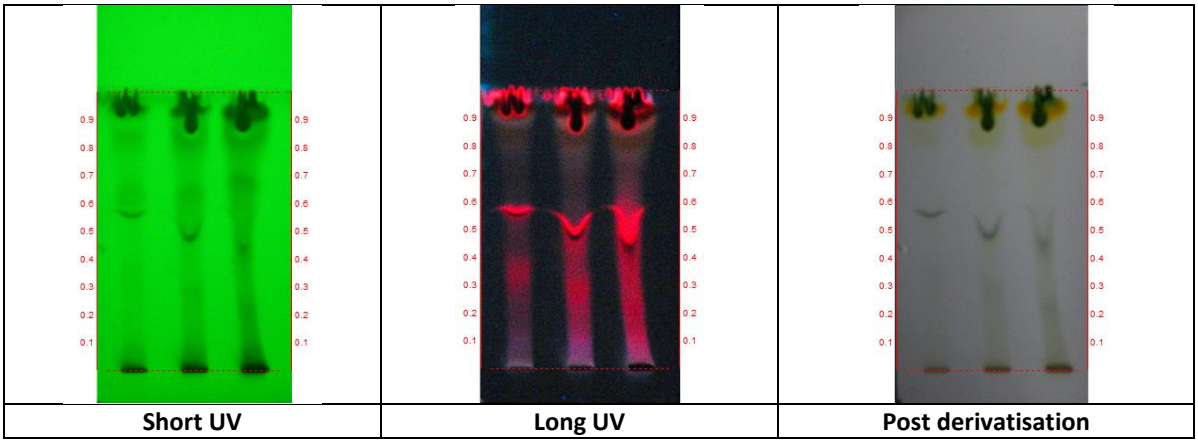


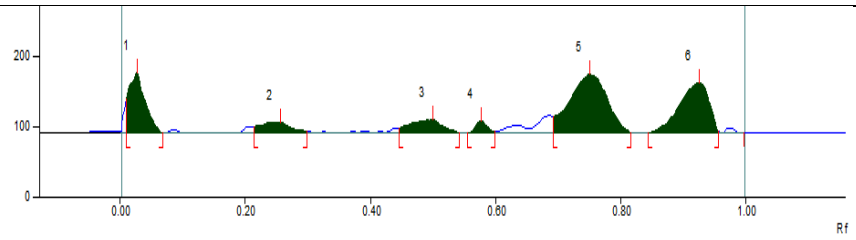
Figure 1: HPTLC photo documentation of methanolic leaf extract of *Streblus asper*

Track 1 - ***Streblus asper*** – 4µl
Track 2 - ***Streblus asper*** - 8µl
Track 3 - ***Streblus asper*** – 12µl
Solvent system –Ethyl acetate: Methanol (9.6: 0.4)

Table 1: R_f value of Methanolic leaf extract of *Streblus asper*

Short UV	Long UV	Post derivatisation
0.24 (Green)	-	-
0.44 (Green)	-	-
0.48 (Green)	**0.50 (F. red)**	**0.48 (Green)**
0.58 (Green)	-	-
0.69 (Green)	-	-
-	-	**0.82 (Yellow)**

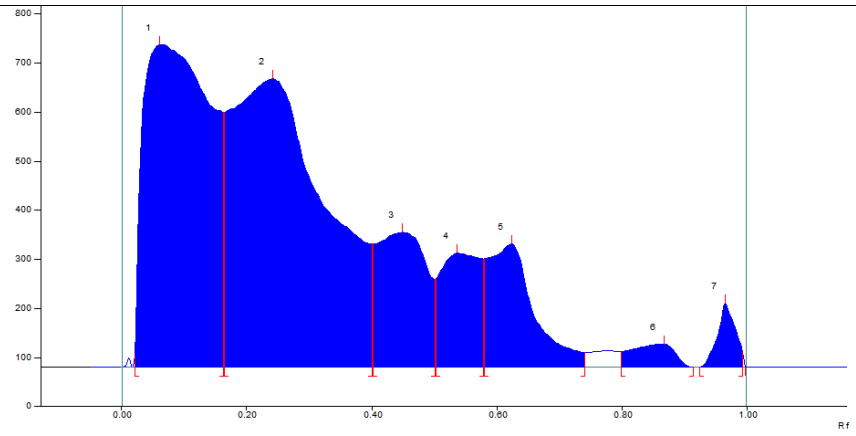
***f- fluorescent**



Track 3, ID: Methanolic extract pf leaf of Streblus asper

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	47.3 AU	0.03 Rf	85.3 AU	29.57 %	0.07 Rf	0.2 AU	1612.7 AU	17.97 %
2	0.21 Rf	8.5 AU	0.26 Rf	14.7 AU	5.08 %	0.30 Rf	1.1 AU	512.7 AU	5.71 %
3	0.45 Rf	6.4 AU	0.50 Rf	18.5 AU	6.41 %	0.54 Rf	0.7 AU	681.3 AU	7.59 %
4	0.56 Rf	0.6 AU	0.58 Rf	17.0 AU	5.91 %	0.60 Rf	1.8 AU	219.1 AU	2.44 %
5	0.69 Rf	23.6 AU	0.75 Rf	82.5 AU	28.58 %	0.82 Rf	0.1 AU	3529.6 AU	39.34 %
6	0.85 Rf	0.0 AU	0.93 Rf	70.5 AU	24.45 %	0.96 Rf	1.6 AU	2417.1 AU	26.94 %

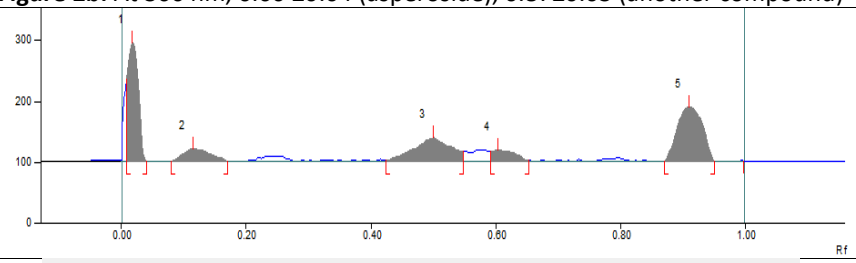
Figure 2a: At 254 nm, 0.50- Strebloside



Track 3, ID: Methanolic extract pf leaf of Streblus asper

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	21.7 AU	0.06 Rf	656.6 AU	30.13 %	0.16 Rf	18.5 AU	50651.1 AU	31.84 %
2	0.16 Rf	518.6 AU	0.24 Rf	587.4 AU	26.96 %	0.40 Rf	50.6 AU	64153.8 AU	40.33 %
3	0.40 Rf	250.7 AU	0.45 Rf	274.3 AU	12.59 %	0.50 Rf	77.9 AU	15456.9 AU	9.72 %
4	0.50 Rf	179.1 AU	0.54 Rf	231.9 AU	10.64 %	0.58 Rf	20.3 AU	10513.7 AU	6.61 %
5	0.58 Rf	220.6 AU	0.62 Rf	250.8 AU	11.51 %	0.74 Rf	30.0 AU	13294.6 AU	8.36 %
6	0.80 Rf	31.5 AU	0.87 Rf	47.3 AU	2.17 %	0.91 Rf	0.1 AU	2360.1 AU	1.48 %
7	0.93 Rf	0.1 AU	0.97 Rf	130.6 AU	5.99 %	0.99 Rf	30.9 AU	2638.1 AU	1.66 %

Figure 2b: At 366 nm, 0.66 ±0.04 (asperoside), 0.87±0.03 (another compound)



Track 3, ID: Methanolic extract pf leaf of Streblus asper

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	135.0 AU	0.02 Rf	193.8 AU	53.90 %	0.04 Rf	0.1 AU	2464.8 AU	31.23 %
2	0.08 Rf	0.2 AU	0.11 Rf	20.2 AU	5.63 %	0.17 Rf	0.2 AU	645.4 AU	8.18 %
3	0.42 Rf	1.5 AU	0.50 Rf	38.7 AU	10.77 %	0.55 Rf	15.8 AU	1652.4 AU	20.94 %
4	0.59 Rf	16.0 AU	0.60 Rf	18.1 AU	5.04 %	0.65 Rf	1.2 AU	510.6 AU	6.47 %
5	0.87 Rf	0.4 AU	0.91 Rf	88.7 AU	24.67 %	0.95 Rf	0.3 AU	2618.2 AU	33.18 %

Figure 2c: At 470 nm, 0.48 ±0.02 – Strebloside

Figure 2: Densitometric scan of Methanolic leaf extract of *Streblus asper*

Light and Dark exploratory test (LDT)

In case of LDT, time spent in light and dark rooms was measured in a two-chambered setup where the animal may freely roam between a brilliantly illuminated open field and a dim corner. In the LDT, animals were treated with standard Diazepam 4mg/kg, low and high doses of the *Streblus asper* Lour extract 200 and 400 mg/kg respectively have shown statistically significant p<0.0001**** results when compared to the control group. Time spent by animals in light chambers is increased in all treatment groups. The higher dose of the test drug has shown better results when compared to standard Diazepam (4 mg/kg). Observations of time spent by animals in each chamber have been given in table 3.

Open field test

A paradigm for evaluating anti-anxiety activity was put out using an open field test. The animals that received *Streblus asper* Lour extract (200 and 400 mg/kg) performed better than those who received normal saline as a control group. Similar results were obtained with animals administered 4 mg/kg of diazepam. High doses of the test have shown comparably better results than standard. Observations on the number of squares crossed by animals and number of readings were given in table 4.

Staircase Method

Using a staircase test on mice, the test medication (*Streblus asper* Lour) was evaluated for its ability to reduce anxiety. Orally administered doses of 200 and 400 mg/kg had a noticeable impact on the quantity of rearing and the number of steps climbed. Diazepam, a standard medication, has a noticeable anxiolytic effect (4 mg/kg). Observations on the number of steps climbed and number of rearings by animals were given in table 5.

Table 2: Effects of treatment groups in tail suspension test model

Sl. No	Body weight (g)	Drug	Dose	Immobility time	
				Before drug administration	After drug administration
1	20	Control	15 ml/kg	73	74.000 ±0.316
2	20	Standard	4 mg/kg	71	158.000 ± 0.707 ****
3	22	Low dose	200 mg/kg	65	108.800 ± 0.374****
4	22	High dose	400 mg/kg	69	141.800 ± 0.374****

Values reported as Mean ± SEM (n=5). The data was analyzed by one-way ANOVA followed by Dunnett's test < 0.0001****:

Table 3: Effects of treatment groups in light and dark exploratory test model

Sl. No	Body weight (g)	Drug	Dose	Time spent (second)			
				Light compartment		Dark compartment	
				Before	After	Before	After
1	24	Control	15 ml/kg	70	71.800 ± 0.490	230	233.000± 0.447
2	24	Standard	4 mg/kg	69	153.400 ± 0.510****	231	147.000±0.316****
3	24	Low dose	200 mg/kg	67	121.800 ± 0.374****	233	178.400±0.678****
4	24	High dose	400 mg/kg	71	288.000 ± 0.707****	224	11.600 ± 0.400****

Values reported as Mean ± SEM (n=5). The data was analyzed by one-way ANOVA followed by Dunnett's test < 0.0001****.

Table 4: Effects of treatment groups in open field test model

Sl.No	Body weight (g)	Drug	Dose	Number of squares Crossed		Number of rearings	
				Before	After	Before	After
1	20	Control	15 ml/kg	72	72.600 ±0.510	8	7.000 ± 0.316
2	20	Standard	4 mg/kg	71	41.400 ± 0.510****	7	2.600 ± 0.400****
3	20	Low dose	200 mg/kg	73	37.200 ± 0.374****	8	2.400 ± 0.245****
4	20	High dose	400 mg/kg	72	26.800 ± 0.374****	8	0.400 ± 0.245****

Values reported as Mean ± SEM (n=5). The data was analyzed by one-way ANOVA followed by Dunnett's test <0.0001****.

Table 5: Effects of treatment groups in staircase test model

Sl. No	Body weight (g)	Drug	Dose	Number of steps Climbed		Number of Rearing	
				Before	After	Before	After
1	24	Control	15 ml/kg	42	42.600 ± 0.510	5	6.000 ± 0.316
2	24	Standard	4 mg/kg	41	22.200 ± 0.374****	6	1,600 ± 0.245****
3	24	Low dose	200 mg/kg	40	13.800 ± 0.374****	5	2.600 ± 0.245****
4	24	High dose	400 mg/kg	42	6.200 ± 0.374****	6	0.600 ± 0.245****

Values reported as Mean ± SEM (n=5). The data was analyzed by one-way ANOVA followed by Dunnett's test <0.0001****.

Table 6: Effects of treatment groups in forced swim test model

Sl. No	Body weight (g)	Drug	Dose	Immobility time	
				Before administration (second)	After administration (second)
1	20	Control	15 ml/kg	48	55.600 ± 0.400
2	20	Standard	4 mg/kg	45	131.400 ± 0.510****
3	24	Low dose	200 ml/kg	46	120.400 ± 0.510****
4	24	High dose	400 ml/kg	49	137.600 ± 0.510****

Values reported as Mean ± SEM (n=5). The data was analyzed by one-way ANOVA followed by Dunnett's test <0.0001****.

Forced swim test (FST)

As compared to the control group, which was given with regular saline, the immobility period in FST was considerably longer after treatment with *Streblus asper* Lour (200 and 400 mg/kg) and Diazepam (4 mg/kg). Observations on immobility time in the forced swim test model have been given in table 6.

DISCUSSION

Most anxiety disorders have an unknown cause, although studies have demonstrated both GABA and serotonergic neurotransmission play a role in the development, manifestation, and treatment of anxiety. It was also found that the alterations in dopaminergic and adrenergic systems lead to anxiety. Despite the plant's widespread conventional use of *Streblus asper* Lour treating a wide range of diseases, there are no reports of scientific examinations of its anxiolytic activity. The current study proved that methanolic leaf extract of *Streblus asper* Lour showed anxiolytic activity in mice in a variety of anxiety-related animal models. These are the most well-established animal models for testing sedative and anxiolytic medicines.

When faced with unfamiliar, unrewarding, or punitive environmental events, anxiety causes a specific type of behavioral inhibition. Animals may become immobilized or suppress a behavioral response as a result of this behavioral inhibition. Development of new anxiolytic drugs requires animal testing that gives a good guide to activity in humans, and much ingenuity has gone into developing and validating such tests. For instance, when placed in an unusual location, rats typically react by standing stationary for a while, exhibiting vigilant behavioral suppression, which may indicate "anxiety" brought on by the strange surroundings. When anxiolytic medications are consumed, their immobility is decreased.

The brightly lighted environment acts as a toxic environmental stressor in the light and dark exploration tests, which prevents mice from acting in an exploratory manner. Reduced entry frequency, duration spent, and rearing behavior in the light box were all considered signs of anxiousness. Reduction reflects an animal's exploratory tendencies, which can be diminished by intense anxiety.

The animal exhibits emotional instability, fear, and anxiety in an open field. Animals that are anxious tend to spend more time on the open field's edges and outer zones. In an open field, measures such as the number of squares crossed, the rearing rate (standing on the hind limb), and the time that is spent in the center square are measured to evaluate activity.

The effectiveness of novel anti-anxiolytic medications as well as their neurobiological mechanisms are frequently detected and characterized using the tail suspension test and the forced swimming test. These animal models are susceptible to different anti-anxiolytic drugs and were based on the helplessness or desperation behavior of some confined and inescapable animals. The current finding supported the previous finding that treatment with a methanolic extract of *Streblus asper* Lour had an anti-anxiolytic-like effect in mice performing the forced swim test and the tail suspension test by considerably lowering the immobility period in comparison to stress.

In numerous laboratories, the stair-case test has been shown to be an easy and trustworthy approach for evaluating anxiolytics. The staircase test was first introduced for the evaluation of anxiolytic activity in mice. Rodents suffer anxiety when placed in a strange setting, which is shown in their increased attentiveness and behavioral activity. Step climbing is said to reflect exploratory or locomotor activity in the stair-case paradigm, whereas rearing behavior is a measure of anxiety. Over the course of a 5-minute period, the number of steps and rearing are counted. To quickly screen mice for anxiolytic action, the test was altered.

In this above mentioned all five animal models *streblus asper* lour has shown satisfactory anxiolytic potential when compared to the standard diazepam. All the results observed in treatment groups were statistically significant (p <0.0001****) when compared to the control treated group. The higher dose of *streblus asper* lour has wide anxiolytic potential when compared to the conventional drug Diazepam.

CONCLUSION

The present study demonstrated that the methanolic leaf extract of *Streblus asper* Lour exhibited dose-dependent anxiolytic activity. The presence of alkaloids, glycosides, reducing sugars, flavonoids, saponins, carbohydrates, and

steroids is established by phytochemical analysis done in previous research. The strebloside and asperoside are the major active constituents that are observed in methanolic leaf extract of *streblus asper* lour, which are responsible for its antioxidant potential and will help in treatment of anxiety disorders. Further, there is a need to isolate, characterize, and screen the active principles for its molecular targets. From the results, it is confirmed that methanolic leaf extract possesses significant anti-anxiety properties and can be used as an anti-anxiety agent. We can suggest our product for further research and development at molecular level.

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