Journal of Applied Pharmaceutical Science 2025. Article in Press
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2025.231418
ISSN 2231-3354

Phytochemical screening and evaluation of antifertility activity of *Streblus asper* leaf extract in female albino mice

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ARTICLE HISTORY

Received on: 03/12/2024
Accepted on: 25/06/2025
Available Online: XX

Key words:
Anti-implantation, abortifacient, estrous cycle, *Streblus asper.*

ABSTRACT

The plant kingdom offers a wealth of biologically active substances with diverse pharmacological effects. India has a long-standing tradition of using medicinal plants for their contraceptive properties, both for females and males. In this study, mice were administered methanolic leaf extract of *Streblus asper* (MESA) at 200 and 400 mg/kg body weight doses alongside 17β estradiol (1 µg/kg body weight) as a standard drug. Also, phytochemical analysis of the leaves was done by using methanol, ethanol, water, and petroleum ether as a solvent. Phytochemical analysis revealed that methanolic extract possesses the highest phytoconstituent and percentage yield, while petroleum ether extract possesses the lowest. Based on that, methanol extract was used for further investigation in this study. The findings revealed that MESA altered the duration of various phases of the estrous cycle of mice. Furthermore, the extract also exhibited dose-dependent anti-implantation and abortifacient effects. This study represents an initial step in verifying the plant's purported antifertility properties.

INTRODUCTION

The rapid population growth in developing countries is straining economic progress and human development, highlighting the need for new contraceptives [1]. Even though there are numerous commercially available synthetic contraceptives available today to manage fertility, most of them have severe adverse effects, which include weight gain, hormonal changes, hypertension, and cancer [2]. Over 80% of people in developing nations rely on affordable traditional plant-based medicines due to the high cost of pharmaceuticals [3,4]. Ayurvedic and traditional medicine systems have been explored for antifertility effects, and herbal medicine has gained global acceptance [2]. Searching for more advanced and effective herbal medications that are entirely reversible, less expensive, and have minimal to no adverse effects is one of the most challenging tasks [5].

Streblus asper Lour., a small tree from the Moraceae family, native to tropical regions including India [6], is traditionally used to treat various ailments such as piles, tuberculosis, leprosy, and fever. Its bark treats fever, diarrhea, and dysentery; its roots address sinusitis and ulcers; and its latex is used for elephantiasis and glandular swellings [7]. Recent studies highlighted its neuroprotective properties [8]. The plant's twigs are also used to clean teeth, earning it the name "toothbrush tree" [9,10]. It is thought that the presence of different chemical elements, such as flavonoids, phenolic acids, and alkaloids, is the reason for the varied pharmacological activity of this plant [11].

The contraceptive activity of *S. asper* has been previously documented in male mice [12]; however, no published reports are available on its contraceptive effects in females. Also, hormonal contraceptives available in the market are widely used by females. However, male hormonal contraceptives are still undergoing clinical trials. Moreover, *S. asper* has been traditionally noted for its use in treating various disorders, including antifertility and abortifacient activities. The Tripura tribes of India used the stem of this plant along with other herbs to induce abortion [13]. Additionally, it has been reported to have long-term contraceptive effects with minimal side effects [14], and its fresh stem is known to facilitate abortion [15].

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The methanolic leaf extract of *S. asper* (MESA) contains various bioactive compounds [11] that may regulate reproductive physiology in female mice, potentially influencing the estrous cycle, implantation, and pregnancy. So, it is necessary to validate the contraceptive activity of *S. asper* in females. If the plant has contraceptive activity in females, it can be used as a candidate for potent herbal contraceptive. Consequently, the current study aims to determine the effect of methanolic leaf extract of *S. asper* on the estrous cycle, implantation, and pregnancy of female mice.

MATERIALS AND METHODS

Plant materials

In April 2022, the leaves of the fully grown plant were acquired from Deomornoi, District Darrang, Assam, India. The plant material received authentication from the Botanical Survey of India (B.S.I.), Shillong, Meghalaya, with the voucher specimen number ID/1383/*S. asper*.

Preparation of extracts

The *S. asper* leaves were collected, cleaned well, and sliced into little pieces. Then, leaves were allowed to air dry in the shade, ground with a mechanical grinder until finely ground, and put in an airtight container. By using a Soxhlet extractor with extractor capacity of 400 ml, powdered leaves (30 g for each extraction) were subjected to extraction with different solvents (300 ml of each solvent) for 20 hours in the ratio of 1:10 (1v of plant material:10v of solvent), namely methanol, ethanol, distilled water, and petroleum ether. After the extraction process, the solvent was removed using a rotary evaporator, resulting in 3.24 g of dried methanolic extract, 2.24 g of dried aqueous extract, 1.31 g of dried ethanolic extract, and 0.078 g of dried petroleum ether extract. After that, the percentage yield was calculated for each solvent. The yield of the leaf extract was then reconstituted with distilled water to get the necessary concentration for every pharmacological test. As the animal study was carried out using methanolic extract due to a higher yield of the leaf extract, methanolic extraction was carried out 5 times to get the required amount of extract for the entire study. On the other hand, aqueous, ethanolic, and petroleum ether extracts of the leaf were prepared only one time to do the phytochemical screening of *S. asper* leaves.

Phytochemical screening

The phytochemical profiles of crude methanolic, ethanolic, aqueous, and petroleum ether extracts of *S. asper* leaves were determined using standard methods. The presence of alkaloids, flavonoids, saponins, steroids, tannins, phenol, reducing sugars, carbohydrate, ketone, and anthraquinone glycosides was assessed following the protocols described by Auwal *et al.* [16]. The screening for glycosides and terpenoids was conducted according to the method outlined by Parekh *et al.* [17] and Wadood *et al*. [18], respectively.

Percentage yield

The percentage yield of the different extracts was determined as the percentage of the extract's weight to the original weight of the dried sample used [19].

$$\text{Percentage yield} = \frac{\text{Weight of dry extract}}{\text{Weight of dry plant material}}$$

The methanolic extract possesses the highest phytoconstituent and percentage yield compared to other solvents, so it was used for further investigation in this study.

Liquid chromatography-mass spectroscopy (LC-MS) analysis

The LC-MS analysis of the leaves of *S. asper* was performed by an LC-MS/MS (Agilent 6410Triple Quad MS-MS). The detection was performed through direct injection mode with an electrospray ionization probe at the positive mode. The capillary temperature was kept at 300°C, while the sample flow rate was 0.4 ml/min. The mass range was selected from 50 to 1500 m/z. The gas flow was maintained at 6 ml/min, and the maximum pressure was set at 400 bar. As a mobile phase, the ratio of 0.5% formic acid in water and acetonitrile was 95.5 for the High-Performance Liquid Chromatography fraction of *S. asper.* The MS parameters for each compound were optimized to ensure the most favorable ionization and ion transfer conditions. They attained the optimum signal of both the precursor and fragment ions by infusing the analytes and manually turning the parameters. The source parameter was identical for all of the analytes.

Experimental animals

A healthy female colony breed groups of five albino mice ($n = 5$) each were kept in conventional laboratory settings with a temperature of 25°C ± 2°C and a 12/12 hours light/dark cycle. The mice were weighed between 25 and 28 g. They were fed a regular pellet diet and given unlimited access to water. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Gauhati University, Guwahati, India (Approval No.: IAEC/Per/2024-24/02-6).

The sample size ($n = 5$ per group) was determined following previously published studies of a similar nature [20] and animal ethics committee guidelines [Organization for Economic Co-operation and Development (OECD) guideline] that recommend using the minimal number of animals necessary to achieve statistically valid results [21]. This approach balances scientific objectives and animal welfare considerations.

Determination of acute toxicity

Albino mice were used to test the acute toxicity of methanolic leaf extract of *S. asper.* Prior to the experiment, the animals were fasted for the entire night, and the proper dose was used in accordance with OECD 425 guidelines, 2022 (OECD, 2022) [21]. The extract was given orally to the mice up to the maximum dose of 4,000 mg/kg/day, and animals were observed for any sign of toxicity. All animals were observed for toxicities such as diarrhea, decreased appetite, lacrimation, convulsion, salivation, lethargy, paralysis, and mortality for different time intervals, i.e., 1, 3, 24 hours, and consecutively for 14 days.

Experimental design

Animals were divided into eight groups, with five mice in each group.

Table 1. Phytochemical analysis of different extracts of *Streblus asper* leaves.

Name of secondary metabolites	Test performed	Methanol	Ethanol	Aqueous	Petroleum ether
Alkaloid	Mayer's test	+	−	−	+
	Dragendorff test	+	−	−	+
	Wagner's test	+	−	−	+
Reducing sugar	Fehling test	+	+	−	−
Saponin	Foam test	+	+	−	−
	Froth test	+	+	−	−
Steroid	Liebermann-Burchard test	+	+	+	+
Terpenoid	Salkowski test	+	+	+	+
Phenol	Ferric chloride test	+	+	−	−
Glycosides	Bontrager's test	+	−	+	−
	Keller Killani test	+	−	+	−
Ketone		−	−	+	−
Carbohydrate	Molisch's test	−	−	+	−
Flavonoid	Alkaline reagent test	+	+	+	−
Tannin	Ferric chloride test	+	+	−	−

(+) = presence; (−) = absence

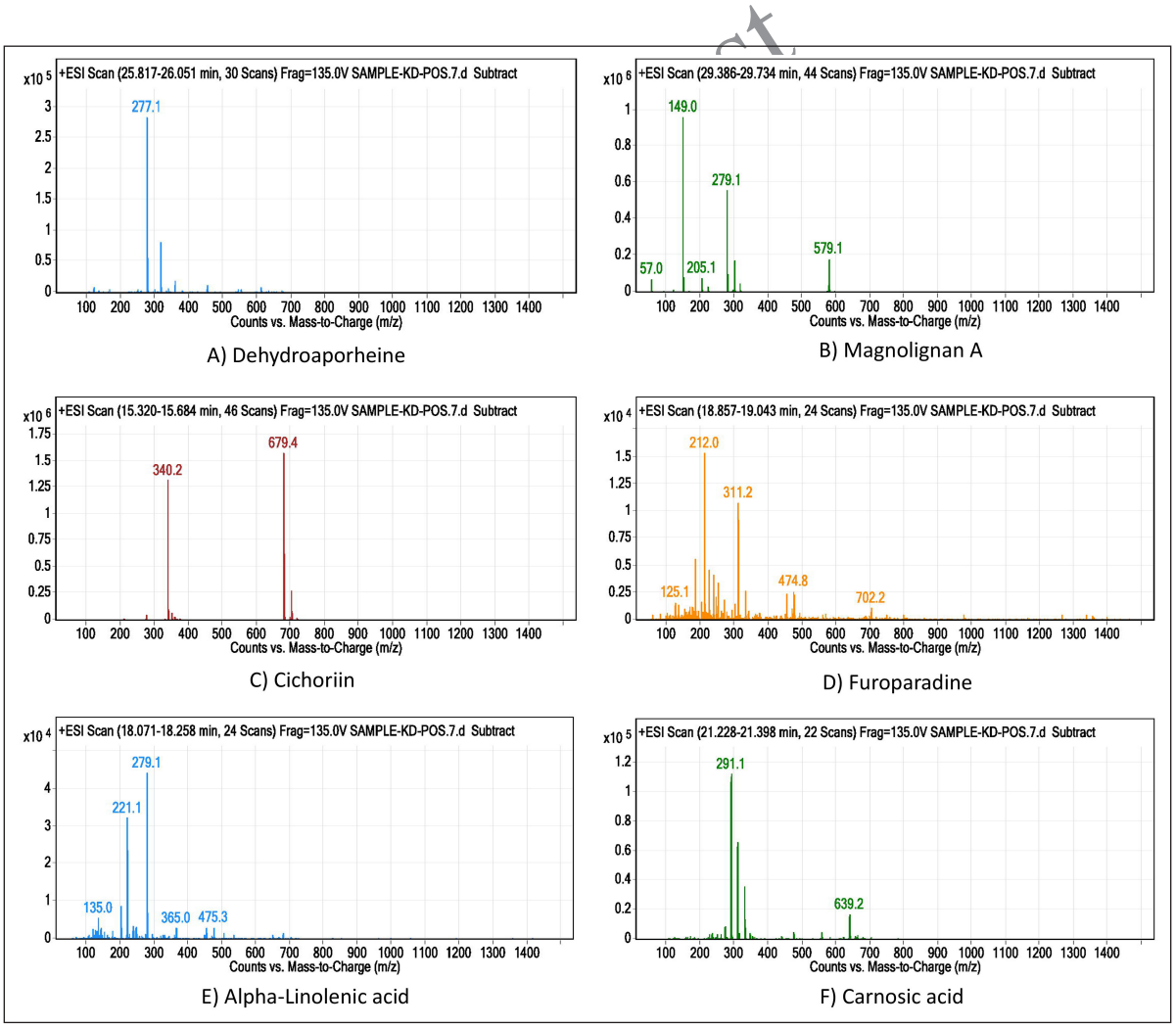

Figure 1. LC-MS chromatogram of probable phytocompounds identified from methanol extract of *S. asper* leaves.

Table 2. List of probable bioactive compounds quantified from methanolic extract of *Streblus asper* leaves by LC-MS.

Sl. No.	Molecular mass (g/mol)	Retention time (min)	m/z	Abundance	Compound name
1	277.3	25.817	277.1	282685.5	Dehydroaporheine
2	300.3	29.734	301.1	174798.3	Magnolignan A
3	340.2	15.320	340.2	1317549.6	Cichoriin
4	311.1	18.857	311.2	10766.8	Furoparadine
5	278.4	18.071	279.1	44209.5	Alpha-Linolenic acid
6	332.4	21.228	332.1	7289	Carnosic acid

Table 3. Effects of MESA on the estrous cycle of mice. Data are expressed as mean ± SEM.

Groups	Duration in days (mean ± SEM)			
	Proestrus	Estrus	Metestrus	Diestrus
Control (8 days)	2 ± 0.31	1.6 ± 0.24	1.4 ± 0.25	3.2 ± 0.20
VC (8 days)	2.1 ± 0.32	1.4 ± 0.25	1.4 ± 0.24	3.1 ± 0.20
MESA 200 (8 days)	1.8 ± 0.20	**2 ± 0.31[a]**	1.2 ± 0.20	**3.2 ± 0.21[a]**
MESA 400 (8 days)	1.6 ± 0.24	**1.6 ± 0.40[a]**	1.8 ± 0.48	**3 ± 0.45[a]**
E2 treated (8 days)	1.4 ± 0.24	**3.6 ± 0.24[#]**	1.8 ± 0.21	**1.2 ± 0.20[#]**
Control (24 days)	7.6 ± 0.25	3.6 ± 0.51	5.8 ± 0.57	7 ± 0.32
VC (24 days)	7.4 ± 0.40	3.8 ± 0.49	5.6 ± 0.51	7.2 ± 0.73
MESA 200 (24 days)	7 ± 0.54	**4.4 ± 0.50[a]**	**2.6 ± 0.40***	**10 ± 0.71*, [a]**
MESA 400 (24 days)	**4.8 ± 0.20*, [a]**	**6.2 ± 0.37*, [a]**	**2.4 ± 0.50***	**10.6 ± 0.24*, [a]**
E2 treated (24 days)	**5.8 ± 0.37[#]**	**9 ± 0.32 [#]**	**3.4 ± 0.40[#]**	5.6 ± 0.68

Data are expressed as mean ± SEM. *Significant difference from the control group. [#]Significant difference from the VC group. [a]Significant difference from the positive control group. $p < 0.05$, $n = 5$.
Note: Values in bold indicate statistically significant differences compared to control and vehicle control groups ($p < 0.05$).

Group I: The control group received standard feed and water ad. libitum

Group II: Received 200 mg/kg body weight of plant extract (for 8 days)

Group III: Received 200 mg/kg body weight of plant extract (for 24 days)

Group IV: Received 400 mg/kg body weight of plant extract (for 8 days)

Group V: Received 400 mg/kg body weight of plant extract (for 24 days)

Group VI: Received 17β estradiol (E2) at a dose of 1 µg/kg body weight suspended in olive oil subcutaneously (for 8 days)

Group VII: Received E2 at a dose of 1 µg/kg body weight suspended in olive oil subcutaneously (for 24 days)

Group VIII: The vehicle control (VC) group received olive oil subcutaneously

Pharmacological Screening

Estrous cycle study

The stained preparations of the animals' vaginal smears were used to examine the estrous cycle every day [22,23]. Every morning, the vaginal smears of the animals in all groups were examined to look for variations in the proestrus, estrus, metestrus, and diestrus phases duration and compared with the estrous cycle of control mice. Smear analysis was conducted using microscopes with 10 and 40× objective magnifications [24].

Antifertility activity

Anti-implantation and abortifacient activity

To assess the anti-implantation activity of the extract, five groups of mice were used having five animals in each group: Group I (control) received standard feed and water; Group II received 200 mg/kg of plant extract; Group III received 400 mg/kg; Group IV received 1 µg/kg of E2 subcutaneously; and Group V (VC) received olive oil subcutaneously. Female mice in the estrus phase were mated with fertile males (2:1). The female mice that showed vaginal plugs were separated, and that day was designated as the first day of pregnancy. The extracts were given orally to the mice from the first to the seventh day of gestation. Light ether anesthetic laparotomy was performed in sterile conditions on the 10th day, and the number of implantation sites was ascertained by examining the uteri [25].

The plant extract was tested in female albino mice for its abortifacient activity according to the method described by Khanna and Chaudhury [26].

Effect on pregnancy and litter size

To evaluate the extract's effect on pregnancy parameters, all animal groups were treated for 24 days. In

the last 5 days, females were mated with fertile males, and those with vaginal plugs were isolated and allowed to carry the pregnancy to term. Mice were weighed daily to monitor weight gain [27]. Litter size, gestation period, body length, and weight of litters were determined using the procedure described by Hastings-Tolsma *et al*. [28].

Statistical analysis

Statistical analysis was performed using MS Office 2017 and SPSS 21. The *t* test and one-way analysis of variance (ANOVA) were employed to evaluate the group differences statistically. Results were expressed as mean ± SE of the mean and differences between means were considered significant at $p < 0.05$.

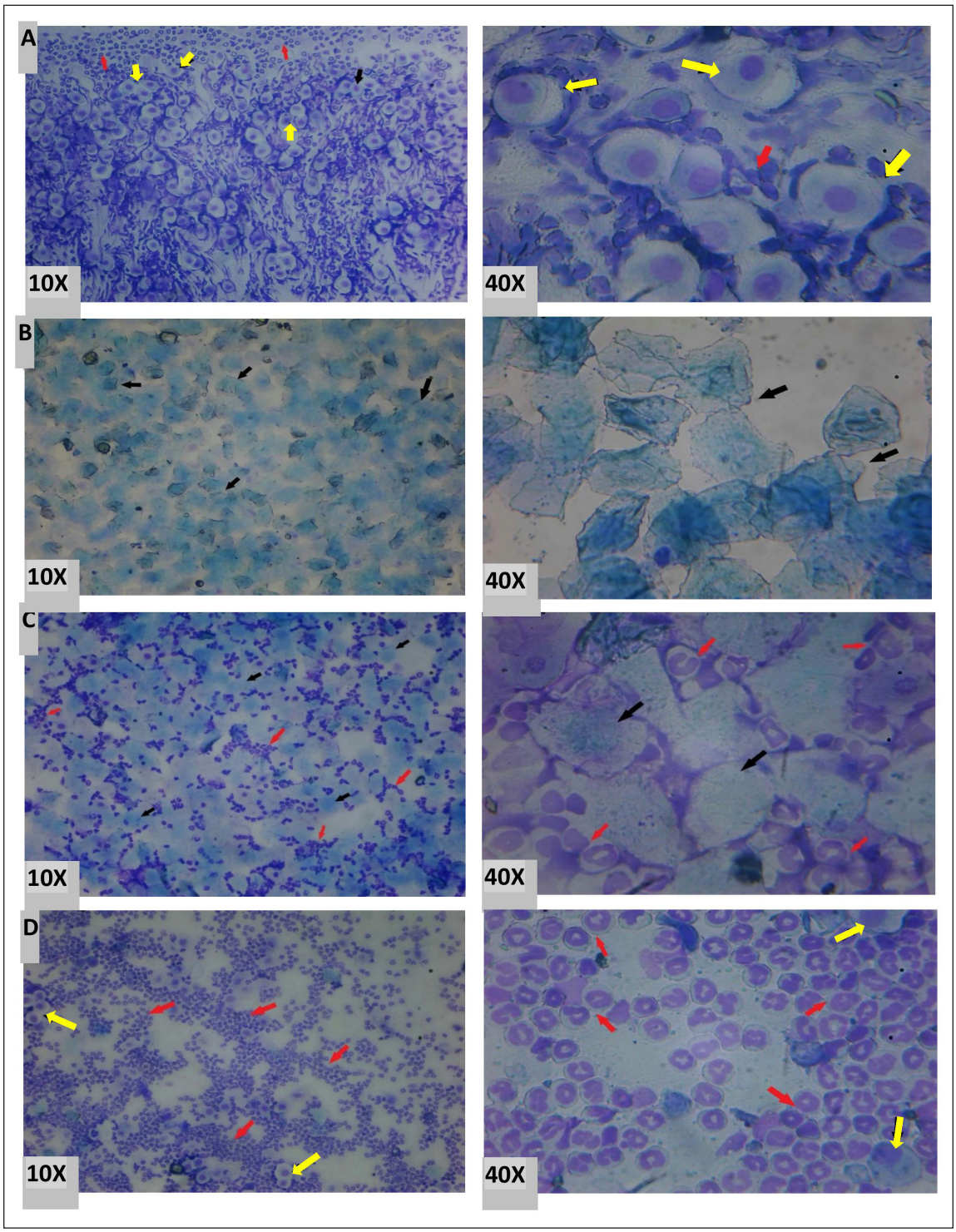

Figure 2. Different stages of the estrous cycle. A- Proestrus phase, B- Estrus, C- Metestrus, D- Diestrus. (→nucleated epithelial cells; →Cornified cells; →Leucocytes).

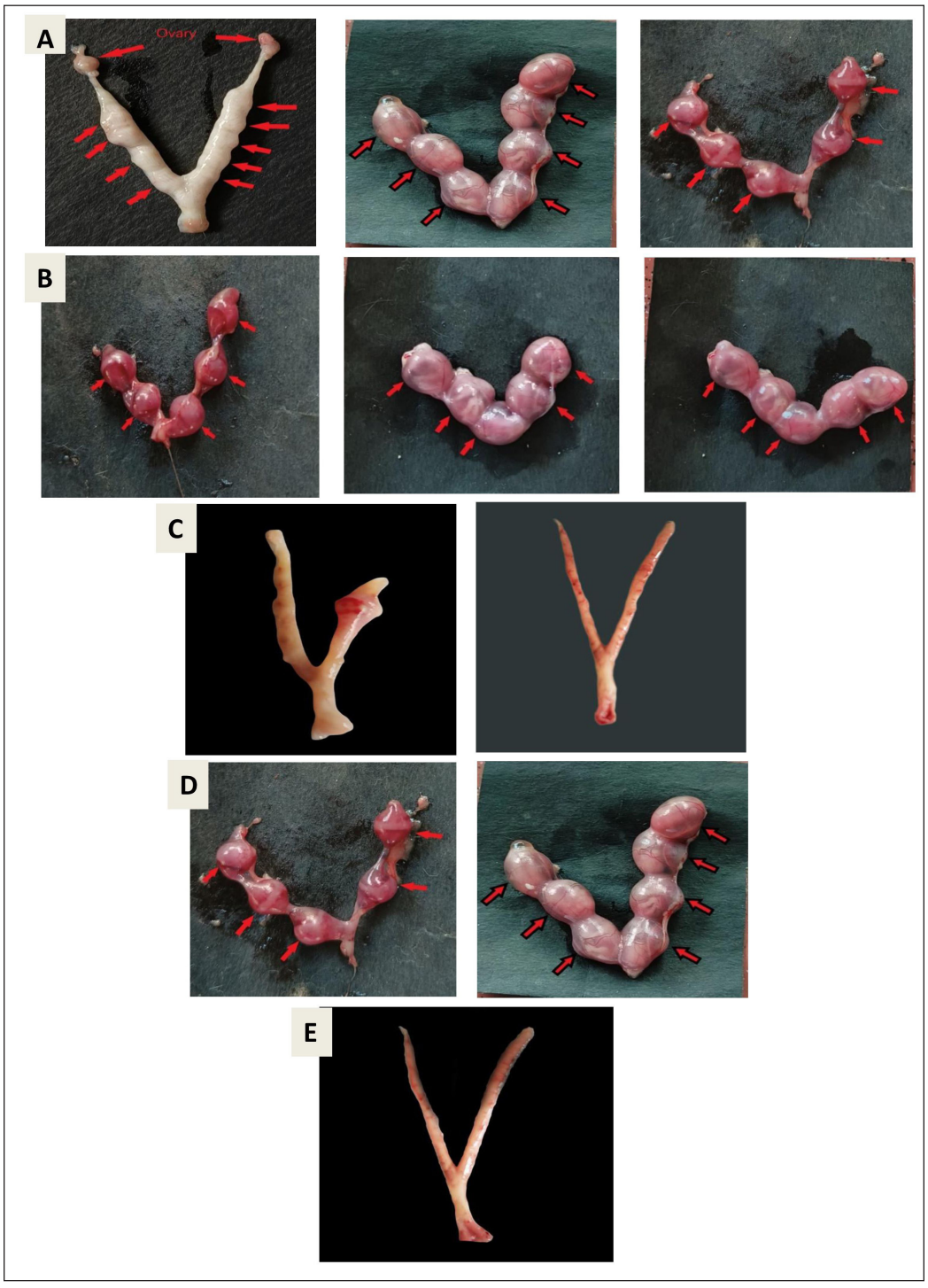

Figure 3. Effect of MESA on the number of implantations on mice. (A) Control group animal showing 9,7 and 5 implants, (B) 200 mg/kg extract treated group showing five implants, (C) 400 mg/kg extract treated group showing 0 implants, (D) VC group (olive oil) animal showing 5 and 7 implants, (E) E2 treated group (1 µg/kg/bw) animal showing 0 implants.

RESULTS

Phytochemical screening

The results obtained in the present study showed that extracts obtained by using different solvents from *S. asper* plant leaves were enriched in phytochemicals such as saponins, flavonoids, alkaloids, steroids, tannins, glycosides, reducing sugar, terpenoid and phenol. Alkaloids, reducing sugars, saponins, and phenols were absent in the water extract. The ethanolic extract lacked alkaloids and glycosides, while the petroleum ether extract lacked reducing sugars, saponins, phenols, glycosides, flavonoids, and tannins. During the study,

it was observed that petroleum ether extract contains minimum phytoconstituents, and methanolic extract contains the highest number of phytoconstituents (Table 1).

Percentage yield

Percentage yield will give an idea about the extractability of the plant studied under different conditions. Among the different solvents examined in the study, methanolic extract showed the highest percentage yield (10.68%), followed by aqueous extract (7.49%), ethanol extract (4.39 %), and finally, petroleum ether extract (0.26%).

Table 4. Effect of MESA on implantation in mice.

Groups	Treatment	No. of observation	No. of implants	Mean	Inhibition (%) of implants
I	Control	1	11	7.8	0%
		2	9		
		3	7		
		4	5		
		5	7		
II	VC (olive oil)	1	9	7	0%
		2	7		
		3	7		
		4	5		
		5	7		
III	MESA 200	1	5	3.8	40%
		2	7		
		3	5		
		4	0		
		5	0		
IV	MESA 400	1	5	2.4	60%
		2	5		
		3	0		
		4	0		
		5	0		
V	E2 (1 µg/kg/bw)	1	0	0	100%
		2	0		
		3	0		
		4	0		
		5	0		

LC-MS analysis

LC/MS chromatogram of methanolic leaf extract of *S. asper* is presented in Figure 1 and identified compounds with their molecular mass, retention time, m/z ratio, and abundance were presented in Table 2. The mass spectral analysis has identified six compounds with various pharmacological activities.

Acute oral toxicity study

The MESA exhibited no toxicity in mice up to 4,000 mg/kg body weight. Consequently, 4,000 mg/kg was considered as the maximum tolerated dose. After administering 4,000 mg/kg orally, no adverse effects such as motor activity alterations, diarrhea, lacrimation, convulsions, or coma were observed, and no fatalities occurred during the 14-day observation period. Following OECD guideline 425, the experimental animals were treated with 400 mg/kg (1/10th) as the high dose and 200 mg/kg (1/20th) as the low dose.

Effects of MESA on the estrous cycle of mice

The impact of *S. asper* methanolic leaf extract on the estrous cycle of mice has been shown in Table 3. Mice administered with 200 or 400 mg/kg body weight of the extract for 8 days showed no visible variations in the duration of the various estrous cycle stages compared to the control group. However, the estrus phase was shorter, and the diestrus phase was longer than the E2-treated group. Over 24 days, all extract-treated and E2-treated groups exhibited longer diestrus and estrus phases and shorter proestrus and metestrus phases than the control group of animals. Various stages of the estrous cycle are depicted in Figure 2A–D.

Anti-implantation and abortifacient activity

The current study revealed a significant decrease ($p < 0.05$) in implantation sites in MESA-treated groups compared to the control group. The 200 and 400 mg/kg doses of MESA showed 40% and 60% anti-implantation efficacy, respectively, with the highest inhibition at 400 mg/kg. The E2-treated group showed 100% inhibition (Fig. 3, Table 4). The extract demonstrated a dose-dependent abortifacient effect, with 47.36% and 91.67% abortion rates at 200 and 400 mg/kg, respectively. As the dose

Table 5. Effect of MESA on resorption index of female mice.

Group	Treatment	No. of mice without implants on day 10/no. of mice used	No. of implants on day 10 (mean ± SEM)	Resorption	%Abortifacient activity
I	Control	0/5	7.8 ± 1.01	0.00 ± 0.00	0%
II	VC	0/5	7 ± 0.63	0.00 ± 0.00	0%
III	MESA 200	2/5	**3.8 ± 1.5***	**1.8 ± 0.73***	47.36%
IV	MESA 400	3/5	**2.4 ± 1.5***	**2.2 ± 1.35***	91.67%
V	E2 (1µg/kg/bw)	5/5	**0**[#]	-	-

*Significant difference from the control group. [#]Significant difference from the VC group. $p < 0.05$, $n = 5$.
Note: Values in bold indicate statistically significant differences compared to control and vehicle control groups ($p < 0.05$).

Table 6. Litter size, body length and weight of litters, and gestation period. Data are expressed as mean ± SEM.

Groups	Mean litter size	Body length (cm)	Body weight (g)	Gestation period (days)
Control	8.4 ± 0.4	2.52 ± 0.04	1.44 ± 0.04	20.2 ± 0.2
VC	8.2 ± 0.37	2.58 ± 0.06	1.42 ± 0.03	20.4 ± 0.24
MESA 200	**3.5 ± 1.19***	**1.86 ± 0.18***	**0.97 ± 0.08***	19.67 ± 0.67
MESA 400	**1.77 ± 0.63***	**1.4 ± 0.1***	**0.7 ± 0.1***	**19.5 ± 0.5**

Data are expressed as mean ± SEM. *Significant difference from the control group. $p < 0.05$, $n = 5$.
Note: Values in bold indicate statistically significant differences compared to control and vehicle control groups ($p < 0.05$).

increased, the number of resorptions increased significantly ($p < 0.05$) (Table 5). There was no vaginal bleeding observed. The 400 mg/kg dose of MESA was substantially more effective ($p < 0.05$) than the control group. Findings are illustrated in Figure 3 and presented in Tables 4 and 5.

Effect on pregnancy and litter size

The study found that oral administration of methanolic leaf extract of *S. asper* reduced litter size in a dose-dependent manner compared to controls. Mice in the control and VC groups had the largest litter sizes that are 8.4 ± 0.4 and 8.2 ± 0.37, respectively (Table 6). Compared to controls, the MESA-treated groups showed a significant ($p < 0.05$) decrease in the litter body weight and total body length on the first day of birth. These effects were more pronounced at the 400 mg/kg dose. However, there was no significant difference in gestation duration between the treated and control groups.

DISCUSSION

Despite the benefits of natural remedies, research on the efficacy of traditional medicines is limited [29]. *S. asper* has been noted for its use in treating various disorders, including its antifertility and abortifacient properties. The Tripura tribes of India utilize its stem, along with other plants, for abortion [13]. It is also reported to prevent pregnancy with long-term effects and minimal side effects [14], and its fresh stem can induce abortion [15].

The study is carried out to evaluate the effects of MESA on the estrous cycle, implantation, and pregnancy in female albino mice. Findings revealed that MESA contains alkaloids, steroids, flavonoids, glycosides, tannins, saponins, and carbohydrates, while petroleum ether extract had the fewest phytoconstituents. The methanolic extract also had a higher percentage yield compared to petroleum ether. Ujagar *et al*. [30] also reported higher yields from methanolic extracts due to better solubility of phytochemicals in polar solvents.

According to previous literature, flavonoids, saponins, and non-steroidal estrogenic compounds, including flavones, flavonones, isoflavonoids, alkaloids, and phenolics are known for their antifertility properties [31]. Soni *et al*. [32] reported that flavonoids, which are present in the stem of *Musa paradisiaca*, might be the source of anti-implantation and abortifacient activity of female rats.

LC/MS analysis revealed the presence of six phytochemical compounds having different pharmacological activities (Table 2, Fig. 1). Among them, cichoriin is a glycoside and coumarin, which possess antidiabetic activity [33] and mitigates oxidative stress [34], carnosic acid is an abietane diterpene which is an antioxidant [35], magnolia A is a kind of biphenyl compound, and biphenyl is an endocrine-disrupting chemical, which may have effects on fertility [36]. Alpha-linolenic acid is a polyunsaturated fatty acid that may reduce pregnancy chances in females [37].

The acute toxicity study revealed that oral administration of the extract at doses up to 4,000 mg/kg did not produce any noticeable adverse effects, such as altered motor activity, diarrhea, excessive tear production, convulsions, or coma, nor were there any fatalities throughout the 14-day observation period. Similarly, research by Kumar *et al*. [38] on brine shrimps indicated that the methanolic extract of *S. asper* was non-toxic in both acute and sub-acute toxicity evaluations. However, in subchronic safety studies, the methanolic extract exhibited mild toxicity, whereas the petroleum ether extract was considered non-toxic. Furthermore, Pandey *et al*. [39] reported that acute toxicity testing of the petroleum ether extract from the stem bark of *S. asper* confirmed its non-toxic nature. However, further studies on sub-acute and chronic toxicity in mice are necessary to establish a comprehensive safety profile.

The estrous cycle in female mammals, including rats and mice, involves recurring changes in reproductive hormones and is characterized by specific cell types in vaginal smears. In rats and mice, this cycle repeats every 4–5 days [40]. In the current study, treated groups showed increased estrus and diestrus durations and decreased proestrus and metestrus durations compared to control groups. These disturbances, including the prolonged diestrus phase, may be due to the phytoconstituents in the extract [41]. The estrous cycle is regulated by ovarian hormones (progesterone and estrogen) and pituitary gonadotropins [42,43], and imbalances in these hormones can cause estrous cycle irregularities [44]. Similar studies have noted that such imbalances can lead to prolonged estrus and diestrus phases and reduced the reproductive cycles [45].

Studies have shown that various plant extracts, such as those from *Dalbergia saxatilis, Mimosa pudica, Garcinia kola*, and *Momordica charantia*, can lengthen the diestrus phase and disrupt the estrous cycle, potentially leading to reduced fertility and altered reproductive functions [46–49]. Because of the significantly increased length of the diestrus phase, ovulation frequency will decrease. In the absence of pregnancy, the extended diestrus is marked by progesterone-producing activities of the corpus luteum [50]. The current findings with S. asper are consistent with these observations, suggesting that the extract may cause similar disruptions in the estrous cycle,

likely due to its anti-inflammatory properties. Saponins in *S. asper*, known for their anti-inflammatory effects, might inhibit enzymes such as COX-2, essential for ovulation and follicular rupture [51–53].

Implantation is the process where an embryo establishes contact with the mother's endometrium to initiate pregnancy. In rodents, implantation generally takes place on the fourth or fifth day of gestation, and disruptions during this time might result in losses during the embryo implantation [54]. The uterine endometrium, influenced by estrogen and progesterone, undergoes changes to become receptive to implantation [55]. Disruptions in these hormonal levels can lead to failed implantation and lead to infertility [56,57]. Chemicals affecting these hormones can make the endometrium non-receptive to the embryo, thereby enhancing antifertility effects [58]. Disruption of the estrous cycle can impair endometrial function, leading to implantation failure [59].

The current study found that MESA contains alkaloids, steroids, flavonoids, glycosides, tannins, saponins, which can potentially prevent pregnancy [60], and also, there are many kinds of literature available on the effect of different plant extracts on implantation and pregnancy of rodents [27,61–64]. Other plants with reported anti-implantation effects include Striga orobanchioides, *Calotropis procera*, and *Lawsonia inermis*. [65–67].

The study observed a dose-dependent abortifacient effect with *S. asper* leaf extract, showing 47.36% and 91.67% abortifacient activity at 200 and 400 mg/kg doses, respectively, indicating that higher doses lead to greater resorption [68] (Table 5). This suggests the extract's potential abortifacient properties, potentially linked to its estrogenic or anti-estrogenic effects [69]. Flavonoids and other phytochemicals in the extract may contribute to these effects, with flavonoids known for their antifertility activity [70–74]. It could also be due to uncontrollably strong uterine contractions, leading to abortion depending on the estrogen levels in the tissues that could be due to uterotonic effects of the combination of enzymes [75]. Previous studies have shown similar effects with flavonoids from other plants, such as *Striga lutea* and *Butea monosperma* [26,76], steroids from *I. trifoliata* [77], and alkaloids from *Graptophyllum pictum* [78]. The contraceptive effects of *S. asper* may be attributed to its alkaloids, steroids, flavonoids, and saponins.

The study showed that treatment with 200 and 400 mg/kg of *S. asper* extract significantly reduced litter size and pup body weight, with the 400 mg/kg dose being the most effective ($p < 0.05$). No litters were observed in the estradiol-treated group due to lack of implantation. Despite significant effects on litter size and pup growth, the gestation duration was unaffected (Table 6). Increased resorption rates indicated disrupted embryo development in post-implantation [25]. The maintenance of pregnancy necessitates a careful balance between estrogen and progesterone, just like in the implantation of embryos in uterine walls, and any disruption in these hormone levels may result in abortion [69,79]. Similar studies, such as those on *Hymenocardia acida* and *Lepidium meyenii*, have also reported dose-dependent impacts on litter size and implantation [80,81]. Overall, MESA exhibited significant anti-implantation and abortifacient effects.

The present findings aligned with previous research done by Vemula *et al*. [12] on the effects of *S. asper* leaf extract in male mice, which demonstrated the antifertility potential of *S. asper* aqueous and methanolic leaf extracts in male mice. Their study reported a significant reduction in sperm count and motility following extract administration, indicating a possible impairment in spermatogenesis, supporting the hypothesis that *S. asper* possessed contraceptive properties. These observed effects may be attributed to hormonal disruptions, particularly in luteinizing hormone and follicle-stimulating hormone, both of which are essential regulators of reproductive cycle. An imbalance in these hormones might lead to disruptions in the estrous cycle and impaired folliculogenesis in female mice, which might be a possible mechanism of action observed in the present study.

CONCLUSION

The current study's findings provide strong evidence for the anti-implantation activity of *S. asper* leaves. The methanolic extract of these leaves disrupted the estrous cycle, prevented implantation in female mice, and induced abortion, which could potentially lead to infertility. These results validate the traditional use of *S. asper* leaves as a contraceptive agent. Consequently, this research suggests that the plant could be a reliable and safe alternative for contraception. Further research is required to identify the bioactive compounds responsible for the extract's anti-implantation and abortifacient effects. These findings underscore the importance of caution when using traditional medicinal plants for reproductive health, highlighting the necessity of investigating reversibility, hormonal effects, and clinical relevance. Ultimately, this study adds to the expanding knowledge of the medicinal applications of plants in fertility management.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

FINANCIAL SUPPORT

A fellowship awarded to Kakali Deka from the CSIR Government of India is acknowledged (File No: 09/0059(15447)/2022-EMR-I.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

Ethical approval details are provided in the 'Materials and Methods' section.

DATA AVAILABILITY

All data generated in this study have been incorporated in this article.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

PLANT IDENTIFICATION AND AUTHENTICATION

The leaves of *Streblus asper* were collected from the Deomornoi area of Darrang district, Assam (India). The Botanical Survey of India, Shillong Meghalaya, authenticated and identified the plant, and an authentication number was given as BSI/ERC/Tech/2023-24/1383.

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How to cite this article:
Deka K, Sutradhar T, Sarma B, Ojah A, Deori S, Borthakur MK. Phytochemical screening and evaluation of antifertility activity of *Streblus asper* leaf extract in female albino mice. J Appl Pharm Sci. 2025. Article in Press.
http://doi.org/10.7324/JAPS.2025.231418