

## ANALGESIC ACTIVITY OF STEM BARK EXTRACTS OF STERBLUS ASPER

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## ABSTRACT

In the present study, ethanolic extracts of *Streblus asper* aerial parts at the doses of 100 and 200 mg/kg was evaluated for the Analgesic activity using the hot plate and acetic acid induced abdominal constrictions in mice. *Streblus asper* aerial parts extract showed significant Analgesic properties in all the models studied.

**Keywords:** *Streblus asper*; Analgesic, Hot plate and Acetic acid induced.

## INTRODUCTION

Pain is the part of a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus. The ascending pathway of pain includes the contralateral spinothalamic tract, lateral pons, mid brain to thalamus and ultimately through the somatosensory cortex of the brain that determines the locations, intensity and depth of pain. Many drugs used to relieve the pain and a few drugs like morphine<sup>1</sup> and aspirin<sup>2</sup> have been significantly used for the last three decades. Most of the pain-relieving chemicals produced pronounced side-effects on the physiology of the body. In the indigenous system of medicine, several plants possess an analgesic property and many investigators screened the plant crude extracts for their analgesic property, viz. *Glaucium grandiflorum*<sup>3</sup> *Basella rubra*<sup>4</sup> *Salpichroa rhomboidea*<sup>5</sup>. *Streblus asper*<sup>6</sup> is a tree known by several common names, including Siamese rough bush, khoi, and toothbrush tree. It is a medium-sized tree native to dry regions in India, Sri Lanka, Malaysia, the Philippines and Thailand. The leaves are 2 to 4 inches long, rigid, oval-shaped, irregularly toothed, and borne on small petioles. Staminate flower heads are spherical with minute flowers. pistillate flowers have longer peduncles. Various parts of the plant are used in Ayurveda and other folk medicines for the treatment of different ailments such as filariasis<sup>7</sup>, leprosy, toothache<sup>8</sup>, diarrhoea, and cancer<sup>9</sup>. It is a well known and documented ethnomedicinal plant. Research carried out using different *in vitro* and *in vivo* techniques of biological evaluation support most of these claims. It has been used in the past as an oral hygiene<sup>10</sup> product and for this reasons it is also known as the toothbrush tree. A twig or stick about eight inches long with a frayed or mashed end to increase the cleaning surface was used as a tooth cleaning aid up until the middle of the twentieth century when the cheap and more practical plastic brush with a toothpaste become common throughout the world.

## MATERIAL AND METHODS

**Drugs and Chemicals:** Analytical grade petroleum ether, ethyl acetate, chloroform, ethanol, glass distilled water, Morphine hydrochloride, Acetic acid, aspirin were used for the study.

**Collection of the plant material:** The stem bark of *sterblus asper* was collected in the month of August - 2007 from Baripada, Mayurbhanj district, Orissa. The fresh plant was identified and authenticated by M.S.Mondal, Additional Director, office of central national herbarium, Botanical survey of India, Howrah, Kolkata. A voucher specimen was deposited in the department (vide letter no. CNH/I-1/(231)/2008/Tech.II/261 dated 19.05.08.

**Extraction:** The stem bark of *sterblus asper* was cut into small pieces, shade dried and pulverized to coarse powder (485 g). The resultant was then subjected for successive extraction with petroleum ether (PeAS), ethyl acetate (EAAS), chloroform (ChAS) and ethanol (EtAS) with Soxhlet apparatus and distilled water (AqAS) with maceration. The extracts were then concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in desiccators.

Table 1: Percentage yield of different plant extracts &amp; its colour

Extract	% Yield	Colour
Petroleum ether (PeAS)	2.92	Brown
Chloroform (ChAS)	0.44	Black
Ethyl acetate (EAAS)	0.15	Black
Ethanol (EtAS)	4.42	Red
Distilled water (AqAS)	2.34	Black

**Preliminary Phytochemical investigation:** The extracts were subjected for preliminary qualitative chemical analysis<sup>[11, 12]</sup> by the standard procedures for identification of various phytoconstituents.

## Pharmacological Screening

**Experimental animals:** Albino rats of either sex weighing 125 – 175 g were purchased from Jai research foundation, Valsad, Gujarat were conditioned in standard macrolon boxes (groups of 5-8 mice or rats/cage) at 20-25°C, and maintained on standard pellet (Purina) and water ad libitum. They were kept in 12/12 h light dark cycle. The institute Animal Ethics committee had approved the experiment protocols and care of animals was taken according to CPCSEA guidelines. The experiment was performed in SSR College of pharmacy, Silvassa, Dadra Nagar and Haveli having registration number 1340/ac/10/CPCSEA, 16<sup>th</sup> April.

**Acute toxicity studies:** A simple method is the 'up and down' or the 'staircase' method. Two mice were injected with a particular dose and observed for a period of 24 h for any mortality. The subsequent doses were then increased by a factor 1.5 if the dose was tolerated, or decreased by a factor of 0.7 if it was lethal. The maximum non-lethal and the minimum lethal doses were thus determined using only about ten mice. Once the approximate LD50 or the range between the maximum nonlethal and minimum lethal doses was found, a final and more reliable LD50 assay was planned using at least three or four dose levels within this range, with a larger number of animals in each group<sup>13</sup>

Analgesic activity<sup>[14,15,16]</sup>

Analgesic activity of alcohol extracts of *sterblus asper* was studied by eddy's hot plate and heat conduction method.

Eddy's hot plate method<sup>[18]</sup>

The animals were divided into six groups of 6 animals each. Group I served as control. Group II served as standard and were injected Morphine hydrochloride (7.5 mg/kg) intraperitoneally. Group III and IV were treated orally with aqueous extract of 200 and 100 mg/kg body weight respectively. The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which

animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The reaction time was noted at 15, 30, 60, 120 min.

#### Acetic acid induced writhing test

In acetic acid induced writhing model [19], albino mice (20-25 g) were divided into three groups each consisting of six animals. One group served as negative control (received 0.6% acetic acid mL/kg), Second group served as positive control (received aspirin 250 mg/kg first & 45 min later given acetic acid), while third group & fourth group received EESA (200 & 100 mg/kg) orally. The writhing movements were observed and counted for 30 min after acetic acid administration.

Statistical analysis: The values were expressed as mean  $\pm$  S.D. Statistical analyses were performed by one way Analysis of Variance (ANOVA) followed by student's t-test.  $p < 0.05$  was considered significant when compared with standard references [17].

#### RESULTS AND DISCUSSION

The present study (Refer Table No. 1 & Table No. 2) has shown that the ethanolic extracts of *Sterblus asper*. At dose 200 mg/kg

exhibited significant analgesic activity being reported for first time, preliminary phytochemical screening showed that the *Sterblus asper* revealed the presence of high sterols, saponin glycosides and flavonoids. The flavonoids are known to possess Anti-inflammatory activity by inhibiting the cyclooxygenase responsible for synthesis of inflammatory prostaglandins. Ethanol extracts exhibited significant dose dependent analgesic activity at the doses tested. The analgesic activity of ethanol extract at 200 mg/kg is comparable with the reference analgesic agent used in this study.

Intraperitoneal administration of *Sterblus asper* aerial parts extracts in the Hot Plate test (100 and 200 mg/kg), and acetic acid induced abdominal constriction Test (100 and 200 mg/kg) showed significant analgesic activity. The plant extract administered intraperitoneally (200 mg/kg) to the mice also showed significant analgesic activity in the Hot Plate test. These results indicate a significant analgesic activity at both dose levels studied. The analgesic activity shown by *Sterblus asper* stem bark extract in various models indicate that the plant extract might possess centrally and peripherally mediated analgesic Properties.

**Table 1: Analgesic Activity (Hot Plate Method) Of *Sterblus asper* stem bark Extracts in Mice**

Groups	Treatment	Dose(mg/kg)	Reaction time in Min.				
			Basal reaction time	15	30	60	120
1	Control	10	5.6 $\pm$ 2.4	5.8 $\pm$ 2.8	5.2 $\pm$ 1.9	5.1 $\pm$ 2.1	5.5 $\pm$ 2.2
2	Standard	7.5	6.0 $\pm$ 2.2	9.3 $\pm$ 2.9	13.8 $\pm$	15	10.2 $\pm$ 2.1
3	EE	200	5.3 $\pm$ 2.7	6.1 $\pm$ 1.8	7.2 $\pm$	11.4 $\pm$ 2.6	6.7 $\pm$ 1.3
4	EE	100	5.3 $\pm$ 2.5	5.7 $\pm$ 1.6	6.4 $\pm$	8.7 $\pm$ 2.2	6.2 $\pm$ 2.3

Results are expressed as Mean  $\pm$  S.D, n=6

#### EE- Ethanol Extract of Stem Bark of *Streblus Asper*

**Table 2: Analgesic Activity (Writhing Reflex Method) Of *Sterblus asper* stem bark Extracts in Mice**

Groups	Treatment	Dose (mg/kg)	Avg. no. of writhing	% Inhibition
1	Acetic acid	10	86.66 $\pm$ 1.63	----
2	ASPIRIN	250	32.66 $\pm$ 0.79	62.39
3	EE	200	57.5 $\pm$ 1.2	31.49
4	EE	100	68.5 $\pm$ 1.4	18.8

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