

Antipyretic and antimicrobial potential of *Sida spinosa* linn. aqueous root extract in rats

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Abstract

Objective: Antipyretic and antimicrobial potential of *Sida spinosa* Linn. Aqueous root extract was evaluated using aspirin and chloramphenicol as standard drugs.

Materials and methods: Roots were collected and extracted with water. The doses of the extract selected were 200 and 400 mg/kg b.w, according to OECD guidelines. Antipyretic potential was evaluated in Brewer's yeast induced pyrexia in rats along with Antimicrobial activity by agar well diffusion technique.

Results: Aqueous extract demonstrated highly significant ($P<0.01$) antipyretic activity during various assessment times (1-5 h) when challenged in yeast induced pyrexia test. Maximum attenuation (65.73% at 3h) was observed at 400mg/kg o.p.

Antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli* and *S. aeruginosa*, was carried out. All microbes were sensitive and activity was concentration dependent.

Conclusion: *Sida spinosa* Linn. root possesses potent antipyretic and antimicrobial activity and has therapeutic potential.

Key Words: *Sida spinosa*, Brewer's yeast suspension 20% w/v, antipyretic activity, antimicrobial activity, Minimum inhibitory concentration, Aspirin.

1. Introduction

The investigation of medicinal properties of various plants attracted an increasing interest since last couple of decades because of their potent pharmacological activities, convenience to users, economic viability and low toxicity.¹ This regained interest to plant-derived medicines is basically due to the multidrug resistance of many antibiotics as well as current widespread perception that green medicine is safe and dependable than the expensive synthetic drugs most of which have adverse effects.²

Pyrexia is caused in response to infection, tissue damage, inflammation and other diseased conditions. High fever often increases disease progression by increasing tissue catabolism, dehydration and existing complaints.³ These conditions all enhance formation of cytokines such as IL-1 β , IL-6 (Interleukin), interferon's, and TNF- α . The cytokines increase synthesis of PGE2 in circumventricular organs in and adjacent to the preoptic hypothalamic area; PGE2, in turn, increases cyclic AMP and triggers the hypothalamus to elevate body temperature by promoting an increase in heat generation and a decrease in heat loss. Aspirin and NSAIDs (Non Steroidal Anti-Inflammatory Drugs) suppress this response by inhibiting PGE2 synthesis.⁴

A number of medicinal plants have been screened for antimicrobial activity in recent years and efforts have been done to identify their active constituents.^{5,6} Many infectious diseases are known to be treated with herbal

remedies. Antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases.⁷

Phyto-botanical and ethno-botanical research have focused for decades on the search for the single “active principle” in plants, based on the assumption that a plant has one or few ingredients which determine its therapeutic effects. But the traditional system of medicine like ayurveda, traditional Chinese medicine or the European pharmacotherapy generally assumes that a synergy of all ingredients of the plants will bring about the maximum therapeutic efficacy.⁸

Recent data suggests that 80% drug molecules are natural products or natural compound inspired.⁹ Ethno botanical survey conducted by C.P. Khare reveals that roots of *Sida spinosa* Linn. are used as nervine tonic and diaphoretic, in debility and fevers.¹⁰

2. Material and Methods

2.1 Drugs and Chemicals:

Aspirin, Brewer’s Yeast, Carboxymethyl cellulose were purchased from Sigma Aldrich, Himedia and S.D Fine chemicals respectively. All chemicals used were of analytical grades.

2.2 Plant material and preparation of extract

Roots of *Sida spinosa* Linn. were collected from surrounding areas of Dharwad and authenticated by Dr. Hebbar, Professor, Government PU College, Dharwad. Roots were washed in running water and chopped into small pieces and shade dried, coarsely powdered and used for extraction. The aqueous extract was prepared by maceration of dried root powder in distilled water for 7 days, at room temperature. The extract was filtered; subjected to rotary flash evaporator under reduced pressure to dryness. The dried extract was stored in desiccators.¹¹

2.3 Preliminary Phytochemical investigation

The preliminary phytochemical investigation of aqueous extract was carried in accordance with the standard methods described in practical pharmacognosy by K.R. Khandelwal.¹²

2.4 Animals

Albino wister rats of either sex weighing 150–200 g were used for the study. The animals were purchased from Sri Venkateshvara Enterprises, Bangalore, India. The animals were maintained under controlled conditions of temperature ($22 \pm 2^\circ\text{C}$), humidity ($50 \pm 5\%$) and 12-h light-dark cycles, fed with commercial stock diet and water, *ad libitum*. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of SET’s College of Pharmacy, Dharwad, India (REG.No.112/1999/CPCSEA) according to prescribed guidelines of CPCSEA, Government of India.

2.5 Pharmacological Evaluation

The suspension of aqueous extract was prepared freshly by using tragacanth (2%) in distilled water and administered orally to experimental animals. The extract was administered at a constant volume of 10 ml/kg for each animal.

2.5.1 Antipyretic Activity

Effect on yeast-induced pyrexia

Group 1	Distilled Water (Normal Control)
Group 2	Brewer’s yeast suspension (10 ml/kg, s.c.) (Positive Control)
Group 3	Brewer’s yeast suspension (10 ml/kg, s.c.) + Extract (200 mg/kg p.o.)
Group 4	Brewer’s yeast suspension (10 ml/kg, s.c.) + Extract (400 mg/kg p.o.)
Group 5	Brewer’s yeast suspension (10 ml/kg, s.c.) + Aspirin (100 mg/kg p.o.)

A 20% w/v suspension of Brewer’s yeast in 0.9% saline was prepared. By insertion of a digital thermometer to a depth of 2 cm into the rectum the initial rectal temperature was recorded. Pyrexia was induced by injecting Brewer’s yeast suspension (10ml/kg, s.c.) into the animals dorsum region. The site of injection was massaged in order to spread the suspension beneath the skin. Immediately after yeast administration, food was withdrawn. The rectal temperature of each rat was measured 17h (normal control) after Brewer’s yeast injection using digital clinical thermometer (Hartmann, Germany). Only animals with an increase in body temperature of at least 0.7°C were used for the study. The

animals received the test compound, standard drug and vehicle by oral administration. Rectal temperatures were recorded at 1, 2, 3, 4 and 5 hours post treatment.¹³

2.5.2 Antimicrobial Activity

Disc diffusion method¹⁴

The antimicrobial activity was assayed against 4-microorganisms namely;

Gram-positive bacteria: *Staphylococcus aureus* ATCC 12598, *Bacillus subtilis* ATCC 6633

Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas* ATCC 25619

Minimal Inhibitory Concentration (MIC) procedure¹⁵

1. The 9 dilutions of each drug have to be done with Brain Heart Infusion (BHI) for MIC.
2. In the initial tube 20 μ l of drug was added into the 380 μ l of BHI broth.
3. For dilutions 200 μ l of BHI broth was added into the next 9 tubes separately.
4. Then from the initial tube 200 μ l was transferred to the first tube containing 200 μ l of BHI broth. This was considered as 10-1 dilution.
5. From 10-1 diluted tube 200 μ l was transferred to second tube to make 10-2 dilution.
6. The serial dilution was repeated up to 10-9 no. dilution for each drug.
7. From the maintained stock cultures of required organisms, 5 μ l was taken and added into 2 ml of BHI broth.
8. In each serially diluted tube 200 μ l of above culture suspension was added.
9. The tubes were incubated for 24 h and observed for turbidity

2.6 Statistical analysis

All data was expressed as mean \pm S.E.M. of 6 rats per group. Statistical analysis was performed using Graph pad prism. Parametric one way analysis of variance (ANOVA) followed by Tukey's post test. The minimal level of significance was identified at $P < 0.05$.

3. Results

3.1 Phytochemical investigation:

The phytochemical investigation of *Sida Spinosa* Aqueous extract showed the presence of phenolics, flavonoids and tannins.

3.2 Pharmacological evaluation

3.2.1 Antipyretic activity

Extract at higher dose (400 mg/kg) has significantly ($P < 0.01$) attenuated hyperthermia induced by Brewer's yeast (Table). The maximum percentage reduction in body temperature by aqueous extract was 65.73% after 3 hours (Figure 1 and 2).

Table 1. Effect of aqueous extract on yeast induced pyrexia

Group & Dose	BBT °C	Rectal Temperature (0C) After 18h of Yeast Injection (Mean \pm S.E.M)					
		0 h	1 h	2 h	3 h	4 h	5 h
Normal Control	36.98	37.14	37.22	37.37	37.57	37.75	37.75
Positive Control	37.25	38.31 \pm 0.33	38.42 \pm 0.24	38.59 \pm 0.16	38.68 \pm 0.18	38.76 \pm 0.27	38.85 \pm 0.23
Extract (200 mg/kg)	36.93	38.31 \pm 0.12	38.00 \pm 0.06 (8.54%)	37.96 \pm 0.05 (23.13%)	37.75 \pm 0.16* (42.65%)	37.93 \pm 0.11 (33.77%)	38.08 \pm 0.13 (28.12%)
Extract (400 mg/kg)	37.37	38.29 \pm 0.08	38.18 \pm 0.10 (29.91%)	38.13 \pm 0.07 (43.28%)	37.86 \pm 0.09** (65.73%)	38.02 \pm 0.07* (56.95%)	38.17 \pm 0.04 (49.37%)
Aspirin (20mg/kg)	37.41	38.21 \pm 0.09	37.93 \pm 0.13 (54.70%)	37.79 \pm 0.13*** (71.64%)	37.58 \pm 0.11*** (87.41%)	37.55 \pm 0.17*** (90.06%)	37.51 \pm 0.19*** (93.12%)

Each value is expressed as mean \pm SEM for 6 animals in each group.

BBT- Basal Body Temperature

*** Significant decrease in rectal temperature $P < 0.001$ with respect to positive control group.

** Significant decrease in rectal temperature $P < 0.01$ with respect to positive control group.

* Significant decrease in rectal temperature $P < 0.05$ with respect to positive control group.

Figure 1: Effect of *Sida spinosa* Linn. Aqueous root extract on yeast induced pyrexia in rats.

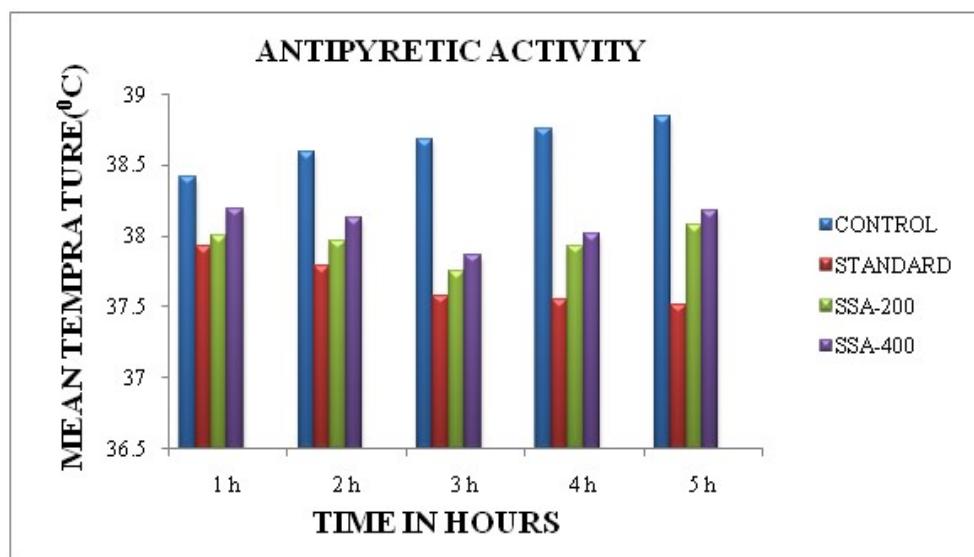
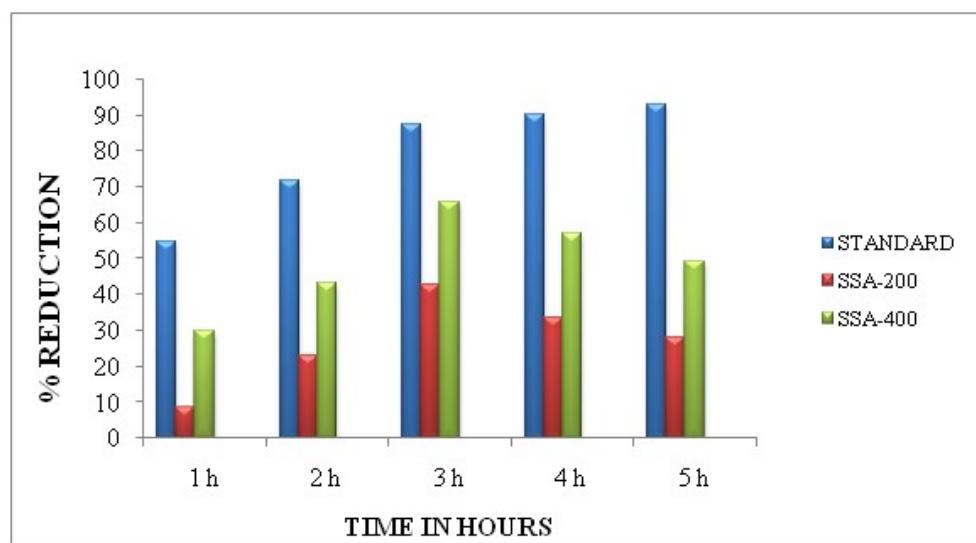


Fig 2: Percentage reduction of rectal temperature by *Sida spinosa* Linn. Aqueous root extract on yeast induced pyrexia in rats



3.2.2 Antimicrobial Activity

3.2.2.1 Agar Well Diffusion Technique

The antimicrobial assay of the extract exhibited *in-vitro* antibacterial activity against Gram-positive and Gram-negative bacteria. The concentration of extract was 10mg/ml. The *Sida Spinosa* Aqueous extracts have shown zone of inhibition of 11 mm at 50 μ l against *Pseudomonas*; resistant at 50 μ l against *E. Coli*; 8 mm at 50 μ l against *Bacillus Sp* 8 mm at 75 μ l against *S. Aureus* (Figure 3, 4, 5 and 6).

Table No 2: Agar well diffusion technique (zone of inhibition)

Test Bacteria	Test Drug	75 μ l	50 μ l	25 μ l	10 μ l	5 μ l	Chloramphenicol (Standard)
<i>Pseudomonas</i>	Aqueous extract	13mm	11mm	R	R	R	>40mm
<i>E.Coli</i>		R	R	R	R	R	15mm
<i>Bacillus Sp</i>		10mm	8mm	R	R	R	26mm
<i>S.Aureus</i>		8mm	R	R	R	R	22mm

(R) Indicates Resistant.

Fig 3: Zone of inhibition of *Sida spinosa* Linn. Aqueous root extract against *Pseudomonas aeruginosa*,



Fig 4: Zone of inhibition of *Sida spinosa* Linn. Aqueous root extract against *Bacillus Subtilis*

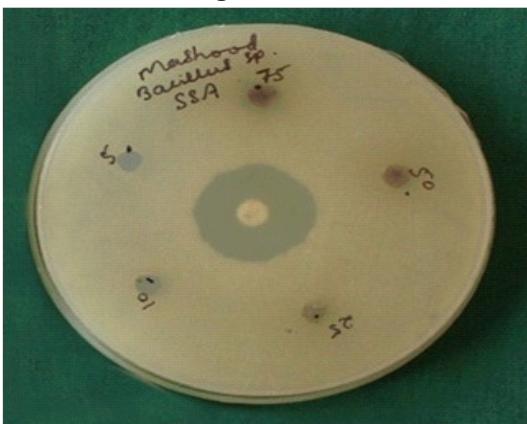
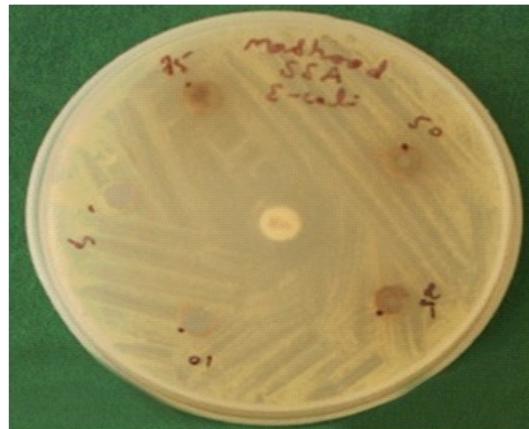


Fig 5: Zone of inhibition of *Sida spinosa* Linn. Aqueous root extract against *Staphylococcus Aureus*



Fig 6: Zone of inhibition of *Sida spinosa* Linn. Aqueous root extract against *Escherichia Coli*



3.2.2.2 Minimum inhibitory concentration (MIC)

MIC values of *Sida Spinosa* Aqueous extract for *E. Coli* and *B. Subtilis* was observed at 0.4 $\mu\text{g}/\text{ml}$. MIC for *S. Aureus* was observed at 1.6 $\mu\text{g}/\text{ml}$, MIC for *Pseudomonas* was observed at 0.8 $\mu\text{g}/\text{ml}$.

Table No. 3 Minimum inhibitory concentration of Aqueous extract of *Sida spinosa*

Test bacteria	Test drug	3.125 $\mu\text{g}/\text{ml}$	1.6 $\mu\text{g}/\text{ml}$	0.8 $\mu\text{g}/\text{ml}$	0.4 $\mu\text{g}/\text{ml}$	0.2 $\mu\text{g}/\text{ml}$
<i>E.Coli</i>	Aqueous extract	S	S	S	S	R
<i>Pseudomonas</i>		S	S	S	R	R
<i>S.Aureus</i>		S	S	R	R	R
<i>B.Subtilis</i>		S	S	S	S	R

(S) Indicates Sensitive while (R) indicates Resistant.

4. Discussion

4.1 Antipyretic Activity

Yeast-induced pyrexia is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermo-regulatory center at a lower temperature (Makonnen et

al. 2003). Fever may be a result of infection, tissue damage and inflammation. According to classical view, fever is induced by inflammatory mediators (i.e cytokines, interleukin-1, interleukin-6, tumor necrosis factor). Within the brain, prostaglandin PGE2 produced by cyclooxygenase (COX-2), is regarded as the principle downstream mediator of fever.

The present study reveals the aqueous extract of *Sida spinosa* linn. root possesses a significant antipyretic effect in yeast provoked elevated body temperature. The aqueous extract at higher dose caused a significant reduction in

body temperature, with the effect being comparable with that of aspirin. Thus, the present pharmacological evidence provides support for the folk fore claim as an antipyretic agent. Aspirin produces antipyretic action, through inhibition of prostaglandin synthesis within the hypothalamus. Therefore it appears that antipyretic action of aqueous extracts of *Sida spinosa* linn. root may be related to the inhibition of prostaglandin synthesis in hypothalamus which is mainly because of presence of flavonoids and phenolics tannins in aqueous extract of *Sida spinosa* Linn. Root.

4.2 Antimicrobial activity

As described above microbial infection is also cause for fever. Lipopolysaccharides found in the outer membrane of Gram-negative bacteria, act as endotoxins induces pyrexia in experimental animals.

Sida spinosa Linn. Root. Extract has shown significant antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*. Almost all the microbes used in the present study were sensitive to aqueous extract except *E. coli*, and showed a potential activity against growth of other gram positive and gram negative bacteria. The activity was concentration dependent against the various micro organisms used. Phenolics, flavonoids and tannins are known to be biologically active because they protect the plant against infection.

Antipyretic activity along with antimicrobial potential clearly indicates therapeutic potential of sida spinosa aqueous extract.

Thus the present study supports the traditional use of *Sida spinosa* Linn. as an antimicrobial.¹⁶

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