

Antimicrobial and Analgesic Activity of *Solanum torvum*

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Abstract: The present investigation was conducted to evaluate antimicrobial and analgesic activities of ethanol extract from *Solanum torvum* (Family: Solanaceae) aerial parts. The bioactive compounds such as glycoside, tannins, sterols, saponins, flavonoids, carbohydrates and proteins are detected in ethanol extract shown promising antibacterial activity against gram positive bacteria viz. *B. subtilis* and *S. aureus*. Analgesic activity was evaluated against both thermal and chemical induced stimuli, which were evidenced from acetic acid induced writhing, tail immersion and formalin induced paw licking test. The assessment of peripheral analgesic effect of the ethanol extracts exhibited a significant percentage inhibition in the writhings which were induced by acetic acid in mice. Similarly test drug significantly increased the latency period in the tail immersion test and the formalin study showed that both the aphasic and tonic pain was blocked by the extract. The overall analgesic effect of ethanol extract (200 and 400 mg/kg body weight, p.o.) was lower than the standard drugs aspirin. The presence of flavonoid compounds in ethanol extract of *Solanum torvum* aerial parts may be responsible for the analgesic effect

Keywords: *Solanum torvum*, Aspirin, Phytochemicals, Antibacterial activity, Analgesic activity.

INTRODUCTION

Solanum torvum Sw, syn. *Solanum ficifolium* Ortega (Family: Solanaceae) is found in moist localities in West Bengal, Bihar, wider part of Odisha and peninsular India. Spreading or sprawling shrubs 2-3 m tall, prickles 3-7 mm long, slightly hooked, laterally flattened, scattered on stems, both leaf surfaces, and main veins, sparse on aged and mature growth, all parts pubescent with stellate hairs, sparse on upper leaf surface, dense on lower surface [1-3,4].

The dried powder of whole plant is used as folk medicine in the treatment for asthma and inflammation [5]. Fresh fruits are used as sedative, expectorant and anthelmintic [6,7]. Leaves are used as sedative and diuretic [8] and also possesses antibacterial, analgesic, antipyretic, anti-malarial and antidiarrheal properties [9-13]. The fruits are reported to have hypoglycemic and antitumor-promoting effect [14-15] and possesses antibacterial, antifungal activity [16]. The leaves of the plant are reported to be use as antiviral, antidiabetic [17-18]. Presence of campesterol, beta-sitosterol, stigmasterol [7,13], chlorogenin, chlorogenon, torvonin A, torvonin B [7, 19-23] and solasodiene [24] in the leaves have been reported earlier.

The present study was under taken to investigate the antimicrobial and analgesic activity of aqueous and hydro-alcoholic extracts of entire plants of *Solanum torvum*. The antimicrobial activities were determined by using disk diffusion assay and Minimal Inhibitory Concentration (MIC) values. The analgesic evaluation was examined using the tail immersion tests and acetic acid-induced writhing method in mice.

MATERIALS AND METHODS**Plant material**

The plant material (aerial parts) was collected from the forests of Paschim-Medinipur district of West Bengal during June 2017 and identified by the taxonomists of the Botanical Survey of India, Shibpur, Howrah, West Bengal, India. A voucher specimen has been kept in our research laboratory for further reference. The collected materials were washed with water and shade dried for one week. The dried plant materials were pulverized using a mechanical grinder to obtain a coarse powder.

Preparation of extracts

The powdered plant material (500 g) was extracted with 1.5 litres of ethanol (90% v/v) for 48 hrs using a Soxhlet extractor. The extract obtained was evaporated under vacuum to remove the solvent

completely and concentrated to obtain a dark greenish semisolid residue (8.48 g).

Preliminary phytochemical tests

Preliminary phytochemical studies of ethanolic extract were performed for determination of major phytochemical constituents using standard procedures [25, 26].

Anti-microbial activity

Ethanolic extracts was tested against a panel of 4 pathogenic bacterial strains including *Escherichia coli* MTCC 1610, *Pseudomonas aeruginosa* MTCC 424, *Bacillus subtilis* MTCC 121, and *Staphylococcus aureus* MTCC 1430 obtained from Indian institute of cholera and Enteric diseases, Kolkata, India.

Animals

Swiss albino mice (20-25 g) and Wistar albino rats (150–250 g) of either sex were maintained in the animal house at Netaji Subhas Chandra Bose Institute of pharmacy, Chakdaha, Nadia, West Bengal, India under standard environmental conditions of temperature (25°C) and light/dark cycles (12/12 h). All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Netaji Subhas Chandra Bose Institute of pharmacy (Regd. No. 1502/PO/a/11/CPCSEA). All standard drugs and ethanolic extract were suspended in normal saline solution using sodium carboxy methyl cellulose (0.5% w/v) for pharmacological studies. All control groups animals received 0.5% w/v sodium CMC in normal saline as vehicle (3 ml/kg body weight, per os) through oral route.

Determination of zone of inhibition

The zone of inhibition of the test samples was performed by disc-diffusion assay as suggested by *Awoyinka et al.* [27]. The dried plant extracts were dissolved in 5 per cent dimethylsulphoxide (DMSO; Merck, Germany) and then in sterile water, to reach a final concentration of 30 mg/ml and sterilized by filtration by 0.22 µm Millipore filters. The media used were Mueller Hinton Agar (HiMedia) for the bacteria. The discs (5 mm in diameter) were impregnated with 10 µl of the extracts (300 µg/disc) at a concentration of 20 mg/ml and placed on the inoculated agar (10^6 CFU/ml). Tetracyclin (30 µg/ml) were served as positive reference standards to determine the sensitivity of the tested microbial strains. Control tests with the solvent DMSO (5%) employed to dissolve the plant extracts were performed for all assays and showed no inhibition of microbial growth. The inoculated plates were incubated at 37°C for a period of 24 hours for bacterial strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organisms. All inhibition assays and controls were made in triplicate.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of aqueous and hydro-alcoholic extracts of *Solanum torvum* with respect to different test microorganisms were determined by broth dilution method [28]. Mueller Hinton broth (HiMedia) was used for the antibacterial study. The extracts dissolved in 1 per cent of DMSO were first diluted to highest concentration (200 mg/ml) to be tested, and then serial two-fold dilution were made in a concentration range from 0.39 to 200 mg/ml in sterile water. For broth dilution, 0.1 ml of standardized suspension of a strain (10^6 CFU/ml) separately was added to each tube containing various extracts at concentrations of 0 (control), 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200mg/ml in the broth medium. The tubes were incubated at 37° C for 24 h for bacterial strains and looked for visible growth after vortexing the tubes gently. The lowest concentration of test extract in a tube that failed to show any visible macroscopic growth was considered as its MIC. Inhibition of proliferation was assessed by optical density measurements (625 nm). The MIC determination was performed in triplicate for each organism.

Acute toxicity study

The acute toxicity studies were conducted on Swiss albino mice as per the OECD guidelines 423, [29] where the test dose limit of 2000 mg/kg, p.o., was used. The test was carried out as suggested by Ganapaty *et al.* [30] and Shivhare *et al.* [31]. Immediately after dosing, the animals were closely observed for the initial 4 h after the administration and then once daily during the following days. The behavioral changes were closely observed for hyperactivity, ataxia, convulsion, salivation, tremors and sleep. They were then kept under observation up to 14 days after drug administration to determine the mortality, if any. One-tenth and one-fifth of the maximum tolerated dose (200 and 400 mg/kg, body weight, p.o.) of the ethanol extract of *Solanum torvum* was selected for analgesic activity studies.

Evaluation of analgesic activity

Acetic acid induced writhing method

The test was performed according to *Sawadogo et al.*[32]. Writhing was induced in mice by single intraperitoneal injection (10 ml/kg) of 0.6% acetic acid. The number of writhings was counted over a 20 min period. Group one served as a control and received only vehicle (3 ml/kg, p.o.), the group two received aspirin (100 mg/kg, p.o.), which was used as reference standard for activity comparison; group three and four received tested ethanol extract of 200 and 400 mg/kg, p.o. respectively. The writhing effect was indicated by stretching of abdomen with simultaneous

stretching of at least one hind limb. The percentage inhibition was calculated [33].

Tail immersion method

Analgesic activity was also checked in Wistar albino rats by the caudal immersion method [34]. The tail withdrawal response was determined by immersing the tail up to the caudal portion (5 cm from the tip) in hot water at a constant temperature of $55\pm 0.5^{\circ}\text{C}$. Group one served as a control and received vehicle (3 ml/kg, p.o.), the second group received aspirin (100 mg/kg, p.o.) used as reference standard for activity comparison; group three and four received ethanol extract of *Solanum torvum* (200, and 400 mg/kg, p.o.) respectively. The reaction time for withdrawal of tail was recorded after 60 min from administration of test compounds. Observation was made at an interval of 30, 60 and 90 mins. The maximum time of observation would be about 60 sec throughout to avoid any tissue damage [35].

Formalin induced paw licking method

The method of Tjolsen *et al.* [36] was used. In formalin induced paw licking, 0.05 ml of formalin (2.5% formaldehyde) was injected into the plantar surface of the rat hind paw, 30 min after treating the rats with ethanol extract (200, and 400 mg/kg, p.o.) and standard drug aspirin (100 mg/kg, p.o.). The time on licking the injected paw by each rat was observed as soon (early phase 0-5 min, post injection) as the formalin was injected and later (late phase 15-30 min). The mean time spent on licking the injected paw in each group was determined. Pain responses were indicated by elevation or favouring of the paw or excessive licking and biting of the paw. An analgesic response or protection is indicated if both paws are resting on the floor with no obvious favouring of the injected paw.

STATISTICAL ANALYSIS

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's-t test. A P-value < 0.05 was considered to be significant. All the values were expressed as mean \pm SEM.

RESULTS

Preliminary phytochemical tests

Preliminary phytochemical screening of the ethanol extract from *Solanum torvum* contains glycoside, tannins, sterols, saponins, flavonoids, carbohydrates and proteins (Table 1).

Anti-microbial activity

The results of antimicrobial susceptibility (Table 2) reveals ethanol extracts have promising

antibacterial activity against gram positive bacteria (*B. subtilis* and *S. aureus*) as compare to gram negative bacteria (*E. coli* and *P. aeruginosa*).

Acute toxicity study

No mortality or morbidity was observed in animals through the 14 day period following single oral administration. Morphological characteristics (fur, skin, eyes and nose) appeared normal. No tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviors such as self-mutilation, walking backward etc. were observed. There was no significant difference in body weights between control and treatment groups. Food and water intake showed daily fluctuations within the range of control animals. This indicates that the ethanol extract from *Solanum torvum* was safe to a single dose of 2000 mg/kg body weight.

Evaluation of analgesic activity

Evaluation of peripheral analgesic effect through acetic acid induced writhing analysis was done on the basis of the average number of abdominal constrictions indicated by the extension of hind paw of animals (mice) during the writhing test (Table 3). The inhibition in writhing of the test extract were observed for 20 min. Tested Doses of 200 mg/kg and 400 mg/kg produced an inhibition of 20.87% and 46.21% respectively when compared with the control group; whereas the standard drug aspirin (100 mg/kg) treated group possess 51.15% inhibition. When the therapeutic activity of the ethanol extract was compared with standard drug aspirin it showed that the observed peripheral analgesic effect for ethanol extract was less than the standard drug aspirin. In the tail immersion method, ethanol extract at 200 and 400 mg/kg body weight, p.o., exhibited significant increase in reaction time up to 60 min after giving thermal stimulus in a dose dependent manner when compared with control group animals (Table 4). Doses of 200 and 400 mg/kg ethanol extract increased the reaction time from 3.9 to 12.2 sec and from 4.0 to 15.6 sec respectively, however the reaction time of standard drug aspirin (100 mg/kg, p.o.) increased from 3.7 to 18.4 sec. In the formalin induced paw licking test, orally administration of ethanol extract from *Solanum torvum* aerial parts at 200 and 400 mg/kg body weight, showed significant analgesic effect, reducing the licking time in both early and late phases (Table 5). The test extract caused significant inhibition of early phase 33.7%, 51.8% and late phase 40.6%, 54.6% at doses of 200 and 400 mg/kg body weight respectively, whereas standard drug aspirin inhibited paw licking 63.4% in early phase and 67.6% in late phase when compared with control group animals.

Table-1: Preliminary phytochemical tests to identify presence of different phytoconstituents in ethanol extract of *Solanum torvum*.

Sl. No.	Test groups	Inference
1	Alkaloids	-
2	Glycosides	+
3	Tannins and phenolic compound	+
4	Steroids and sterols	+
5	Triterpenoids	-
6	Saponins	+
7	Flavonoids	+
8	Carbohydrates	+
9	Proteins and amino acids	+

(+): Present; (-): Absent.

Table-2: Antibacterial activity of ethanol extracts of *Solanum torvum* against bacterial strain

Microorganism	Diameter of inhibition zone (mm)		MIC (mg/ml)
	Ethanol extract	Tetracyclin	Ethanol extract
E. coli	12.1 ± 0.2	32.0 ± 0.7	100
P. aeruginosa	13.2 ± 0.5	34.3 ± 0.8	200
B. subtilis	28.8 ± 0.2	35.0 ± 0.3	6.25
S. aureus	30.6 ± 0.9	34.6 ± 0.2	12.5

Note: The control disc used for solvent had no zone of inhibition, so there data was omitted from the above data. Inhibition zones including the diameter of the paper disc (5 mm). Results are expressed as the mean ± SEM of triplicate measurements.

Table-3: Analgesic activity of ethanol extract of *Solanum torvum* by acetic acid induced writhing in mice

Treatment	Dose	Avg. no. of writhing	Percentage Inhibition (%)
Control (vehicle treated)	3 ml/kg, p.o.	31.14±2.62	-
Aspirin	100 mg/kg, p.o.	15.21±1.24**	51.15
Ethanol extract	200 mg/kg, p.o.	24.64±2.11*	20.87
Ethanol extract	400 mg/kg, p.o.	16.75±1.17**	46.21

Values are expressed as mean ± S.E. (n=6). All columns are significant using ANOVA; *P<0.05, **P<0.01 when compared to control; Dunnet's t-test.

Table-4: Analgesic effect of ethanol extract of *Solanum torvum* by tail immersion test in rats

Treatment	Dose	Reaction time after administration (sec)			
		0 min	30 min	60 min	90 min
Control (vehicle treated)	3 ml/kg, p.o.	3.6±0.1	3.8±0.2	4.0±0.2	4.0±0.1
Aspirin	100 mg/kg, p.o.	3.7±0.1	7.8±0.1**	11.2±0.1**	18.4±0.2**
Ethanol extract	200 mg/kg, p.o.	3.9±0.1	6.0±0.1*	9.3±0.2**	12.2±0.3**
Ethanol extract	400 mg/kg, p.o.	4.0±0.2	7.2±0.1**	10.8±0.3**	15.6±0.2**

Values are expressed as mean ± S.E. (n=6). All columns are significant using ANOVA; *P<0.05, **P<0.01 when compared to control; Dunnet's t-test.

Table-5: Analgesic activity of ethanol extract of *Solanum torvum* by formalin induced paw licking in rats

Treatment	Dose	Response (time spent in licking) (sec)	
		Early phase within 0-5 min	Late phase within 15-30 min
Control (vehicle treated)	3 ml/kg, p.o.	83.4±2.4	109.1±2.0
Aspirin	100 mg/kg, p.o.	30.5±1.1** (63.4%)	35.3±1.4** (67.6%)
Ethanol extract	200 mg/kg, p.o.	55.3±2.0** (33.7%)	64.8±2.0** (40.6%)
Ethanol extract	400 mg/kg, p.o.	40.2±2.1** (51.8%)	49.5±1.5** (54.6%)

Values are expressed as mean ± S.E. (n=6). All columns are significant using ANOVA; *P<0.05, **P<0.01 when compared to control; Dunnet's t-test.

DISCUSSION

Medicinal plants were the potent source of human health due to the presence of active phytochemical compounds that are responsible for its various pharmacological activities. On the basis of the results obtained the ethanol extract of *Solanum torvum* rich in phytochemical constituents. The presence of various secondary metabolites such as glycoside, tannins, sterols, saponins, flavonoids, carbohydrates and proteins were believed to exhibit the antimicrobial properties and confirmed their antimicrobial efficacy against selected pathogens.

Ethanol extract of *Solanum torvum* aerial parts protected against both thermal and chemical induced stimuli, which were evidence from tail immersion, acetic acid induced writhing and formalin induced paw licking test.

The assessment of peripheral analgesic effect of the ethanol extract exhibited significant percentage inhibition in the writhings which were induced by acetic acid in mice at both the tested doses when compared with the control group. The percentage inhibition of writhings indicated the pronounced peripheral analgesic effect in the context of visceral pain which was comparable to the standard drug aspirin (100 mg/kg, p.o.) within 20 min of test [37].

The tail immersion test is another thermic pain model, which assesses the way an animal responds to moderate continuous pain generated by a tissue [36]. Thermic painful stimuli are known to be selective to centrally but not peripherally acting analgesic drugs [38].

The formalin test is sensitive to NSAIDs. This test has two different phases; the early phase which may be due to direct effects on nociceptors and the late phase which is due to an inflammatory response partly mediated by prostaglandins and can be inhibited by NSAIDs [39]. The activity of ethanol extract of *Solanum torvum* aerial parts was observed in both the phase at the doses of 200 and 400 mg/kg body weight, p.o., and the results of the formalin study, showed that both the aphasic (early phase) and tonic pain (late phase) were blocked by the extract. The formalin test was conducted to distinguish analgesic from anti-inflammatory properties [40]. It was found that ethanol extract, as well as aspirin, exerted a marked analgesic activity in the late phase of the formalin test, suggesting an effect on acute inflammation.

The overall analgesic effect of ethanol extract (200 and 400 mg/kg body weight, p.o.) was lower than the standard drugs aspirin. The presence of flavonoid compounds in ethanol extract of *Solanum torvum* aerial parts may be responsible for the analgesic effect [35,41-42].

The ethanol extract of *Solanum torvum* aerial parts possesses antimicrobial and analgesic activities. Thus, it may require further studies to better understand the mechanism of such action scientifically.

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