



Analgesic, antioxidant and antibacterial activity of *Smilax zeylanica* linn. (family - smilacaceae)

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Abstract

The ethanol extract of the leaves of *Smilax zeylanica* Linn. (Family- Smilacaceae) was subjected to pharmacological investigation to ascertain analgesic, antioxidant, and antibacterial activity. Phytochemical analysis of the extract indicated the presence of reducing sugars, tannins, saponins, gums, steroids, alkaloids, and flavonoids. The ethanol extract showed statistically significant analgesic activity ($P < 0.001$) in acetic acid induced writhing test in Swiss-Albino mice at the doses of 250 and 500 mg/kg-body weight. The extract showed free radical scavenging activity in DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay. In quantitative assay, the extract exhibited DPPH radical scavenging activity with the IC_{50} value of 30.93 $\mu\text{g/mL}$. The extract showed antibacterial activity with the zone of inhibition ranging from 5.39 to 9.21 mm and 9.87 to 12.55 mm against all the tested bacterial strains at the doses of 250 and 500 $\mu\text{g/disc}$, respectively, in disk diffusion assay. The results tend to suggest that the extract might possess some chemical constituents that are responsible for analgesic, antioxidant, and antibacterial activity.

Key words: *Smilax zeylanica*, Smilacaceae, writhing test, 1,1-Diphenyl-2-picrylhydrazyl (DPPH)

Introduction

Smilax zeylanica Linn. (Family- Smilacaceae), local name: Kumarilata, Indian smilax, is a perennial climbing shrub which is locally used as medicinal plant in the treatment of various major and minor ailments. It is widely found in the different South Asian countries, namely, Bangladesh, India, Sri Lanka, Myanmar, Malaysia, and Nepal.

Traditionally, roots and leaves of *S. zeylanica* are used as a substitute for the official drug, Sarasparilla, in the treatment of venereal diseases; decoction is applied for rheumatism, pain in the lower extremities, sores swellings, and abscesses, and also used in the treatment of dysentery (1).

In the previous study, alcohol and aqueous extracts of roots and rhizomes of *S. zeylanica* have shown potential antiepileptic activity (2). *In vitro* antioxidant and HPTLC studies on roots and rhizomes were also conducted and reported (3). Phytochemical research has reported that it contains 1-3% steroidal saponins, phytosterols, starch, resin, sarsapic acid, and minerals (4). Leaves and roots contain diosgenin (5). Roots also contain large amounts of tannin, saponin, 31-norcycloartenol, beta-sitosterol, parillin, phenolic acid, and potassium nitrate. The saponin, on hydrolysis, yields the sapogenins, sarsasapogenin, asparagenin, and sapogenin (6).

In this project work, an attempt was made to justify the traditional uses as per scientific experiments. Moreover, using various standard qualitative chemical tests, the presence of reported compounds were detected.

Upon sufficient literature survey, it is found that a little work has been performed to evaluate the rationale for the uses of this plant in traditional medicine of Bangladesh. In the present study, we therefore tried to evaluate the analgesic, antioxidant, and antibacterial activity of the ethanol extract of leaves of *S. zeylanica*.

Materials and Methods

Sample collection and extraction

The leaves of *S. zeylanica* were collected from Jessore, Bangladesh, and identified by the experts at Bangladesh National Herbarium, Mirpur, Dhaka (Accession no.: DACB 35551). The leaves were shade dried. After sufficient drying, the leaves were cut into small pieces, and then slashed to coarse powder with the help of mechanical grinder. The powder was stored in a suitable container to avoid any possible fungal attack.

Cold extraction technique was applied for extracting leaves. About 120 mg of powder was extracted by maceration for 7 days with 500 mL of ethanol accompanying regular shaking and stirring. The extract was filtered off with clear cotton plug to remove plant debris. The solvent was evaporated at room temperature with an electric fan to get the dried crude extract (yield value 13.4%). After drying, the crude extract was stored in refrigerator at 4°C.

Test Animals and Pathogens

Swiss-Albino mice of either sex (20-25 gm) were collected from animal resources department of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) and were used for the current experiment. The animals were kept in the polypropylene cages and provided with standard rodent foods (ICDDR, B formulated). The animals were acclimatized in animal house, Pharmacy Discipline, Khulna University under the standard laboratory condition (relative humidity 55-60%, room temperature 25±2° C, and 12 hours light: dark cycle) for period of 7 days prior to the pharmacological experiment.

Antibacterial assessment was performed by using both Gram-negative and Gram-positive bacteria like *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi*, and *Staphylococcus aureus*. These pathogens were supplied by ICDDR, B.

Chemicals, Reagents and Standard Drugs

Acetic acid and ascorbic acid were purchased from Merck, Germany. 1,1-Diphenyl-2-pyrcrylhydrazyl

(DPPH) was obtained from Sigma-Aldrich, USA. Loba Chemie Pvt Ltd, India supplied Tween-80. Solvents were of analytical grade. Diclofenac sodium was obtained from Beximco Pharmaceuticals Ltd, Bangladesh.

Phytochemical Tests

The crude extract was subjected to preliminary phytochemical screening for the detection of major functional groups present in the extract (7). The extract showed the presence of reducing sugars, tannins, saponins, gums, steroids, alkaloids, and flavonoids.

Evaluation of Analgesic Activity

The analgesic activity of the sample was studied using acetic acid induced writhing test in mice (8,9). Experimental animals were randomly selected, and divided into four groups denoted as control, positive control, and test group I and II consisting of six mice in each group. Control group received 1% Tween-80 in distilled water at the dose of 10 mL/kg body weight, and positive control group received diclofenac sodium at the dose of 25 mg/kg body weight. Test group I and II were treated with the test sample at the doses of 250 and 500 mg/kg body weight. All the treatments were provided in oral route. A thirty minutes interval was given to ensure proper absorption of the administered treatments. Then acetic acid solution (0.6%) was administered via intra-peritoneal route to each animal as writhing inducer. An interval of 5 minutes was given for absorption of acetic acid. Then number writhing was counted for 10 minutes. Percent inhibition of writhing was calculated and compared with the control group.

In Vitro Antioxidant Activity Test

Antioxidant activity of the ethanol leaves extract was estimated by using stable free radical DPPH (1,1-Diphenyl-2-picrylhydrazyl) both qualitatively and quantitatively (8-11).

Qualitative Analysis

Thin Layer Chromatographic (TLC) technique was applied for qualitative assessment (8). TLC plates

were developed with non-polar, medium polar, and polar solvent systems to resolve compounds of different polarities. The TLC plates were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH (yellow on purple background) radical was observed for the period of 30 min and noted.

Quantitative Analysis

The anti-oxidant potential of the ethanol extract was estimated on the basis of their scavenging activity of the stable DPPH radical (8). At first 2.5 mg extract was mixed with 25 mL of ethanol to prepare 100 µg/mL solution of the extract as stock solution. Another six concentrations of sample were prepared by serial dilution method. These concentrations were 50, 25, 12.5, 6.25, 3.13, and 1.57 µg/mL. Then 1 mL of solution of each concentration was taken into test tubes designed for each concentration. 3 mL of 0.004% DPPH solution was then added to each test tube, and kept for 30 minutes at dark place to allow any reaction that is to be occurred. After 30 minutes, absorbance was measured by UV spectrophotometer at 517 nm. Ascorbic acid was used as standard. Percent inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \left[\frac{(\text{Absorbance of control} - \text{Absorbance of sample or standard})}{\text{Absorbance of control}} \right] \times 100$$

IC₅₀ value was determined from % inhibition vs. concentration graph.

Antibacterial Activity

Disc Diffusion Assay

Disc diffusion assay method was used for the assessment of antibacterial activity of the ethanol leaves extract of *S. zeylanica* against a number of Gram-positive and Gram-negative strains (12). Sterile blank discs (BBL, Cocksville, USA) were saturated with the test extract at the doses of 250 and 500 µg/disc using micropipette. Test extract was prepared using ethanol at the desired concentrations. Control discs (blank) were prepared using ethanol. Both sample and control discs were dried. Sample containing discs, standard antibiotic discs (Kanamycin 30 µg/disc, Oxoid Ltd, UK) and control discs were placed in Petri dishes containing nutrient

agar medium seeded with the test pathogens using sterile forceps. Then Petri dishes were transferred into incubator and incubated at 37 °C for 16 h. After incubation, zone of inhibition was measured using digital slide calipers (12, 13).

Statistical Analysis

Student's *t*-test was used to determine significant differences between the control and test group. Results were considered as statistically significant when $P < 0.05$.

Results

Phytochemical Tests

The ethanol extract was subjected to different qualitative phytochemical tests for detection of different biologically active chemical groups and the results are summarized in the Table 1.

Phytochemical groups	Results
Reducing sugars	+
Glycosides	-
Tannins	+
Saponins	+
Gums	+
Steroids	+
Alkaloids	+
Flavonoids	+
Tannins	+

+ = Presence - = Absence

Table 1: Phytochemical tests of *S. zeylanica* leaves

Acetic Acid-Induced Writhing Test

The extract showed 29.67% ($P < 0.01$) and 59.86% ($P < 0.001$) inhibition of writhing in dose dependent manner at the doses of 250 and 500 mg/kg body weight respectively, which was highly comparable to diclofenac sodium 75.44% ($P < 0.001$) at the dose of 25 mg/kg body weight.

see Table 2.

DPPH Scavenging Activity

The leaves extract exhibited DPPH radical scavenging activity with the IC_{50} value of 30.93 µg/mL, which was highly comparable to the ascorbic acid showed IC_{50} value of 14.18 µg/mL.

see Graph 1.

Antibacterial Activity in Disc Diffusion Assay

The extract showed antibacterial activity with the zone of inhibition ranging from 5.39 to 9.21 mm and 9.87 to 12.55 mm against all the tested pathogens at the doses of 250 µg/disc and 500 µg/disc, respectively.

see Table 3.

Discussion

The ethanolic extract of the plant (leaves) delineated some promising phytochemicals, namely, reducing sugars, tannins, saponins, gums, steroids, alkaloids, and flavonoids.

For assessing *in vivo* analgesic activity in mice, more purposely peripheral analgesic activity, acetic acid induced writhing model in mice is most extensively used. In this model, peripherally performing analgesic activity of the sample is assessed by inducing writhing through the sensitization of locally active peritoneal receptors by the release of endogenous substances. Generally, acetic acid liberates several endogenous substances like serotonin, bradykinins, histamine, prostaglandins (PGs), and substance P which are responsible for inducing pain by stimulating nerve endings. Locally active peritoneal receptors activate the abdominal constrictions response (14). The abdominal constriction response induced by acetic acid is a responsive procedure to estimate peripherally acting analgesics (15). The most credible pathway of peripherally acting analgesics may be the embarrassment of prostaglandins (PGE_2 and PGE_{2a}) synthesis (16).

The presence of saponin, tannins, reducing sugars, gums, flavonoids, and alkaloids in the

ethanol extract of *S. zeylanica* may be accountable for the investigated activities, because it is well established that these phytochemicals are responsible for a wide range of bioactivities (16-21).

In vitro antioxidant activity study illustrates that *S. zeylanica* must contain some promising antioxidant compounds. This activity was assessed by most widely used DPPH scavenging assay model. In this model, free radical, DPPH is converted to stable DPPH-H by accepting electron, or hydrogen radical, and deep violet colour of DPPH is converted to light yellow colour.

The DPPH radical contains an odd electron that is detected by UV spectrophotometer at 517 nm against blank, and this absorption decreases due to the development of its non-radical form, DPPH-H, upon reduction with an antioxidant (22). DPPH radical scavenging activity of the extract was in concentration dependent manner that was strongly comparable to the standard antioxidant ascorbic acid. Flavonoids may be responsible for this antioxidant activity which was revealed by the phytochemical investigation of the ethanolic extract (23,24).

Antibacterial activity of the ethanol leaves extract of *S. zeylanica* was investigated against all the tested Gram-positive and Gram-negative bacterial strains in disk diffusion assay based on its traditional uses in the skin diseases (25). By this method, only polar compounds present in the extract may be assessed because nonpolar compounds do not diffuse in water used in the preparation of agar media (25). So the polar compounds may be responsible for the ascertained antibacterial activity of the extract. But macrodilution and tube dilution assay can be good choice to assess both polar and nonpolar antibacterial compounds of the plant extract.

Conclusion

The leaves extract of *S. zeylanica* demonstrated potential analgesic, antioxidant, and antibacterial activities which justify its uses in traditional medicine, and stipulate further investigations to classify underlying active compounds responsible for

bioactivities as well as its mechanism.

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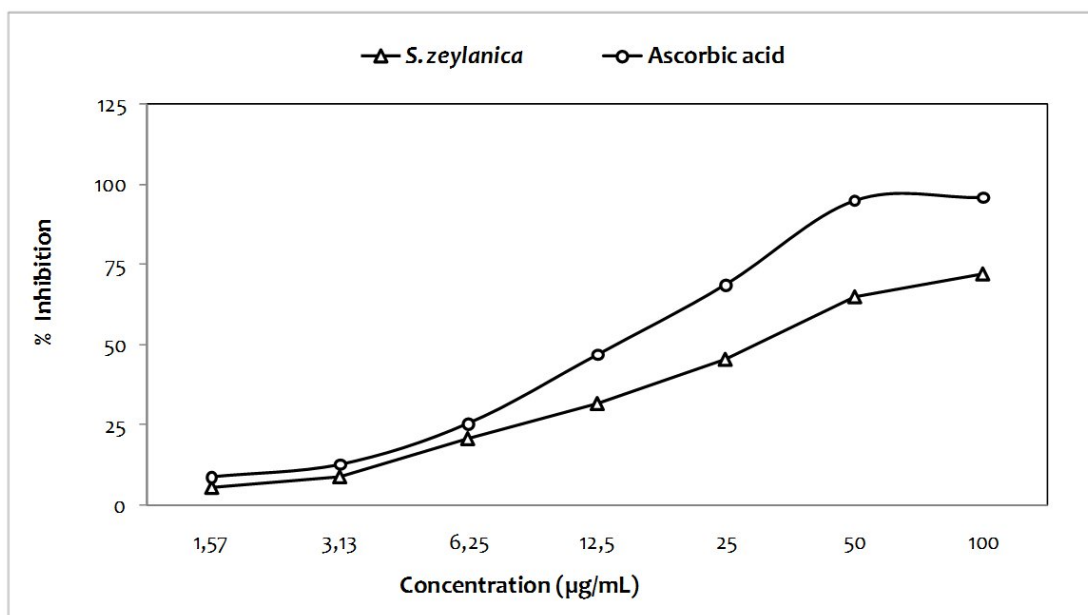


Figure 1: DPPH scavenging activity of *S. zeylanica* leaves

Treatment (n = 5)	Dose (mg/kg)	No. of Wriths	% Inhibition
Control	---	50.2 ± 3.85	---
Diclofenac sodium	25	12.33 ± 1.42**	75.44
Extract	250	35.31 ± 1.17*	29.67
	500	20.15 ± 1.21**	59.86

Table 2: Effect of *S. zeylanica* leaves on acetic acid induced writhing in mice

Values are expressed as mean ± SEM, SEM=Standard error of mean

*: $P < 0.01$; **: $P < 0.001$ vs. control, Student's t-test.

Bacterial strains	Type of bacteria	Diameter of zone of inhibition (mm)			
		Blank	Kanamycin (30 µg/disc)	Extract (250 µg/disc)	Extract (500 µg/disc)
<i>Escherichia coli</i>	Gram (-)	-	24.65	5.39	9.87
<i>Shigella dysenteriae</i>	Gram (-)	-	26.12	8.57	11.19
<i>Salmonella typhi</i>	Gram (-)	-	25.03	8.74	10.89
<i>Salmonella paratyphi</i>	Gram (-)	-	24.34	9.21	12.55
<i>Staphylococcus aureus</i>	Gram (+)	-	27.68	7.45	11.05

Table 3: Antibacterial activity of *S. zeylanica* leaves in disk diffusion assay