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Exploring the anti-inflammatory and antimicrobial potential of *Smilax zeylanica* extract through *in vivo* and *in vitro* analysis

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Abstract
Plants have long been used as supplies of food, building materials, textiles, spices, colours, and medicines. Most rural communities have traditionally relied heavily on the traditional healing system to maintain both physical and mental health. The bioactive components from the leaf extract of *Smilax zeylanica* L. demonstrated strong anti-inflammatory and antibacterial action in the current study. The leaf extract had a maximum inhibition of protein denaturation with 78.65% at 150 µg/ml concentration, compared to the standard drug aspirin which had an inhibition of 67.69% at 150 µg/ml. The *in vivo* anti-inflammatory analysis also showed reduction in edema percentage with increasing in the concentration of test extract over time. The *in vitro* antimicrobial activity of leaf extract against *S aureus, E coli* was evaluated at various concentrations (1, 2 and 4 mg). The maximum zone of inhibition was observed against *S. aureus* as compared to *E. coli*. Total phenolic (TPC) and total flavonoid contents (TFC) of the crude extract were also determined by Folin–Ciocalteu's phenol reagent and by aluminium chloride method respectively.

Keywords: Medicinal plants, anti-inflammatory, antimicrobial, *Smilax zeylanica,* S. *aureus*

Introduction
Plants have long been used as supplies of food, building materials, textiles, spices, colours, and medicines. Most rural communities have traditionally relied heavily on the traditional healing system to maintain both physical and mental health. Ayurveda, Siddha, Unani, and Traditional Chinese medicine are recognised to use plants as an essential part of their own traditional medical systems (TCM). Residents of rural areas all over the world frequently use plant-based medicines. Many Southeast Asian nations, including India, Nepal, Malaysia, China, Bangladesh, and Pakistan, uses medicinal plants to cure a variety of health conditions [1, 2]. Indigenous communities' traditional use of plants represents both cultural and biodynamic characteristics with great pharmaceutical potential to treat a wide range of illnesses [3].

About 300–350 species constitute the monocotyledon family Smilacaceae, which includes the genus Smilax. One of the species is *Smilax zeylanica* L. Smilax is the most significant medicinal species with a distribution throughout tropical India and prevalence in forests [1].The phytoconstituents dioscin "spirostanol triglycoside" and plant steroids like smilagenin and sarsapogenin are reportedly present in Smilax species. *Smilax zeylanica* roots contain the steroidal saponin glycoside diosgenin

With a woody stem and angular branches, *S. zeylanica* is a sizable, scandent, dioecious climbing shrub. Occasionally, the plant has prickles for defence. Umbellate, dioecious flowers that are a greenish-white colour.1-2 seeds are present in the globose berry's fruit, which ripens to a crimson colour. This perennial climbing plant is often employed for rheumatism and pain in the lower extremities, as well as against venereal diseases, skin conditions, ulcers, swellings, and abscesses [4]. In many regions of the world, the plant *S. zeylanica* is used in place of sarsaparilla. A potential alternative source for the Ayurveda medicine chopachine is *Smilax zeylanica* [5]. Many bioactivities, including antibacterial [6], antioxidant [7], cytotoxic [7], analgesic [6], antiepileptic [8], antipyretic [9], anticonvulsant [9], antidiabetic [10], cytoprotective [11] properties were reported from the plant. The goal of the current study was to evaluate the phytoconstituent and anti-microbial of an ethanolic extract of dried and powdered *S. zeylanica* leaves.

Materials and Methods

Collection of plant material, authentication, drying and storage

The leaves of *Smilax zeylanica* L. were collected from Dibrugarh district of Assam, India in the month of March, 2022. The taxonomical identification was done at Guwahati University, Vide Accession no GUBH19913. The leaves were washed thoroughly to remove any foreign particles and then cut into pieces and dried partially under sunlight and partially under shade for a week. The dried leaves were grinded to a coarse powder and stored in an airtight container free from moisture.

Preparation of extract

50gm of dry leaves powder was packed within a thimble and continuously extracted with ethanol until the extraction was finished after 72 hours. The solvent was eliminated using distillation procedure, and the concentrated extract was dried using a rotary evaporator under reduced pressure at a temperature of 37 °C, yielding a weight of 37.80 g. The extract had a sticky consistency and had a dark green colour [1].

Phytochemical analysis

Phytochemical analysis was done to detect the various constituents present in the plant extract [2].

Reagents and chemicals

potassium hydroxide, hydrochloric acid, potassium acetate, catechin, ferrous ammonium sulphate, di-sodium hydrogen phosphate, aluminium chloride, Trichloro acetic acid, tannic acid, quercetin, ethylenediamine tetra acetic acid (EDTA) and ferric chloride were purchased from Loba Chemie Pvt. Ltd. Gallic acid and Folin-Ciocalteu were purchased from Sigma-Aldrich USA and Loba Chemie Pvt. Ltd respectively. All the chemicals used in the experiment were of analytical grade.

Organoleptic evaluation

The appearance, smell, taste, and touch of the plant leaves were characterized by its sensory properties. *Smilax zeylanica* L.'s Macroscopical characteristics were determined by using a basic 10X microscope, where significant structural features were observed [3].

Microscopic analysis of fresh leaf

A fresh leaf of *Smilax zeylanica* L. was taken and cleansed with water. A transverse section of the leaf was taken and treated with KOH to get rid of fatty substances, colouring pigments and other materials. It was then stained with safranin dye and observed under a light microscope Lx300, LABOMED Inc [4, 5].

Quantitative standards

According to the procedure described in the Indian Pharmacopoeia, different quantitative parameters such as total ash, acid-insoluble ash, water-soluble ash, extractive values, and loss on drying were determined and examined. Each investigation was carried out three times, and their means and standard errors of the means were calculated [3].

Determination of total phenolic content

The total phenolic concentration of leaf extract was determined by Folin-Ciocalteu assay by using gallic acid as a standard. For the test, 0.5 ml of leaf extract, 0.25 ml of Folin-Ciocalteu reagent and 3 ml of distilled water were taken in a test tube and mixed properly. 1 ml of 7.5% Na_2CO_3 was added to the above mixture after 5 mins, and incubated for 1.5 hr in dark condition at room temperature. Additionally, distilled water was taken to prepare a reagent blank in parallel. The absorbance was checked at 760 nm by using UV-Visible spectrophotometer compared to reagent blank. The total phenolic compounds in the leaf extract was measured in milligrams of gallic acid equivalent (mg GAE/100 g) per 100 g of sample. All the samples were analysed in triplicate [6].

Determination of flavonoid content

The flavonoid content in the leaf extract was determined by aluminium chloride assay. For the test, 0.5 ml of leaf extract was mixed with 2 ml of distilled water in a test tube. Each test tube contained 0.15 ml of 5% $NaNO_2$ and 0.15 ml of 10% $AlCl_3$ was mixed after 5 minutes. The reaction mixture was added with 1 ml of 1 M NaOH and the volume was adjusted to 5 ml with distilled water. The absorbance was checked at 510 nm by using UV-Visible spectrophotometer. The amount of flavonoid content in each sample was expressed as mg of quercetin equivalent per 100 g of sample (mg QE/100 g sample), using quercetin as the standard [6].

Animal husbandry

Swiss albino mice weighing between 25–30 g were used in this study. The mice were housed at the animal house facility of NETES Institute of Pharmaceutical Science, Mirza, India. The animals were kept in separate polypropylene cages with free access to standard pellet food and water ad libitum. The animals were grouped and kept at an ambient room temperature of 22°C±3 °C and relative humidity of around 40% - 60% with 12 hrs light-dark cycle. The approval for the animal experiment was taken from Institutional Animal Ethics Committee with the approval nos. NIPS/IAEC/2022/35 and the experiment was performed as per CCSEA guidelines.

Evaluation of *in vivo* anti-inflammatory effect

The anti-inflammatory activity of the ethanolic extract of *Smilax zeylanica* was checked by carrageenan induced inflammation in mice paw method. 20 nos of mice were taken randomly and divided into four groups. Group I marked as control and given distilled water. Group II as standard which received diclofenac sodium (10 mg/kg), Group III and Group IV as test which received ethanolic extract of *Smilax zeylanica* at a dose of 250 and 500 mg/kg body weight, 1% carrageenan was injected to the right hind paw of each animal after 30 mins of oral administration of the extract. The volume of paw edema was measured at 0, 1, 2, 3, and 6 hrs using Plethysmometer (Orchid Scientific, Mumbai, India) after carrageenan administration. For comparison, the left hind paw was taken as a control with no inflammation. The percentage of increase in paw volume was calculated by using the formula-

$$\% \text{ of Inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, V_c is paw volume of control and V_t is paw volume of tested animal.

Evaluation of *in vitro* anti-inflammatory activity

In a test tube 0.2 ml of egg albumin (fresh hen's egg), 2.8 ml of phosphate buffered saline having pH 6.4 and 2 ml of varying concentrations (50, 100, 150 µg/ml) of *Smilax zeylanica* extract were added to make a total volume of 5 ml.

Distilled water was taken as control. The mixture was incubated at 37±2 °C in a BOD incubator (Inco Laboratory, India) for 15 min and then heated for 5 min at 70 °C. The mixture was cooled, and the absorbance was checked at 660 nm by using UV-Visible Spectrophotometer (Shimadzu, Japan). Acetyl salicylic Acid at a concentration of (50, 100, 150 µg/mL) was used as reference. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ of inhibition} = \frac{(Abs\ control - Abs\ test)}{Abs\ control} \times 100$$

Antimicrobial activity

Test microorganisms

Strains of S. *aureus* and E. *coli* were used as test organisms in the analysis. The species were obtained from Nemcare Superspeciality Hospital, Guwahati, Assam, and India. The microorganisms were transferred to nutrient broth (Hi Media, Mumbai) and kept at 37 °C for 12–14 hr.

Disc-diffusion method

The *in vitro* antimicrobial activity of the leaf extract was done by disc-diffusion technique [10, 11]. Using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The media was prepared by autoclaving and poured into the sterile petriplates. The plates were allowed to solidify and with a loop the inoculum was transferred to the plate. 6 mm size sterile filter paper discs were prepared and dipped in different concentrations of ethanolic extract of *Smilax zeylanica* 1, 2 and 4 mg/disc). The discs were placed on the petri plates and covered. The plates were incubated at 37 °C at BOD incubator for 24 h. Gentamycin was taken at a concentration of 10 µg/disc as a positive control and put on the plate. After the incubation is over the plates were taken out and their zones of inhibition were measured with a scale.

Results and Discussion

Organoleptic evaluation

The climber *Smilax zeylanica*, leaves measured 13.5 X 9.5 cm in length and diameter. The collected leaves were fresh and had a dark green outer surface, which became brown when dried. The flavour and smell of the leaves were distinctively musky. The images of the leaves are given in the Fig. 1.

Fig1: Fresh leaf of *Smilax zeylanica* L.

Microscopic analysis

The transverse section *Smilax zeylanica* L. leaf showed the presence of xylem, phloem, as shown in Fig. 2 and the powder microscopy revealed the fibres in Fig. 3 and stomata in Fig. 4.

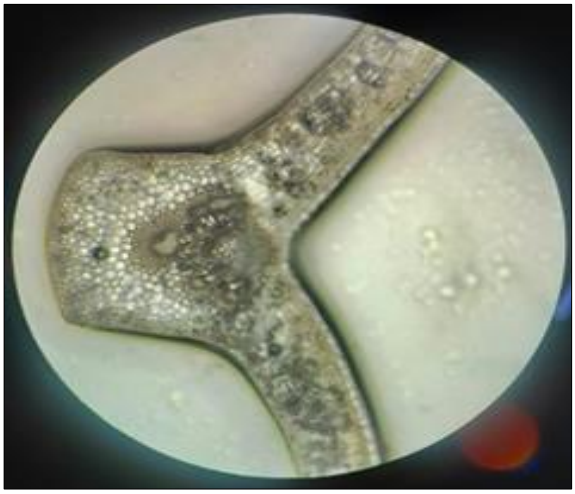

Fig 2: Xylem and phloem

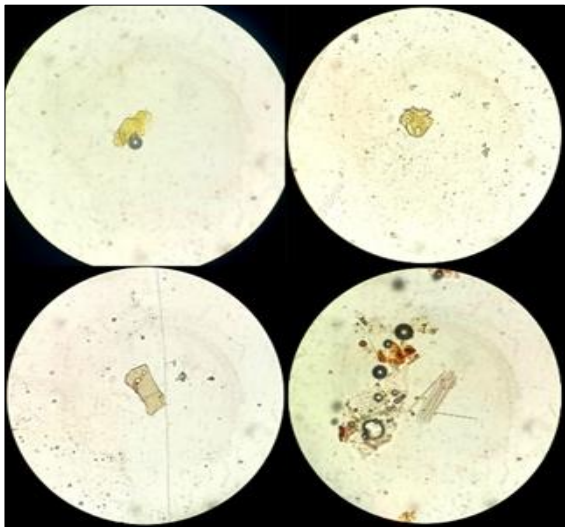

Fig 3: Fibres

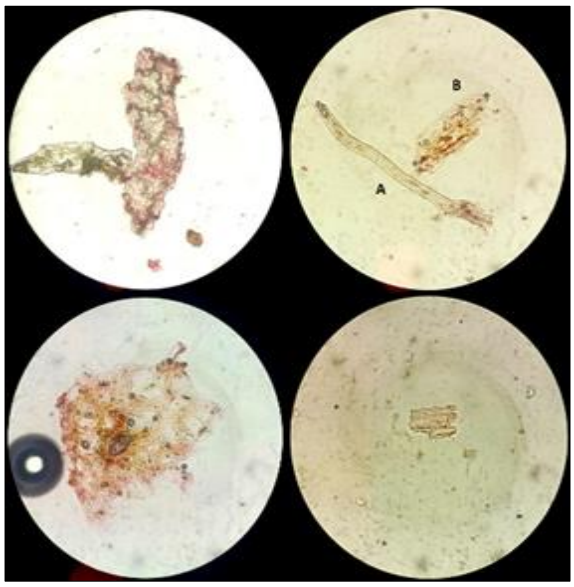

Fig 4: Stomata

Preliminary phytochemical screening

The extract obtained via Soxhlet method was semi-solid in consistency with a distinctive musky odour. Test for alkaloid, amino acid, protein, carbohydrate, flavonoid, phenol, saponin, glycosides, anthraquinone and triterpenoids were done and listed in the Table 1 for their presence or absence [2, 5].

Table 1: Phytochemical screening of *Smilax zeylanica*

Plant constituent	Test	Ethanolic extract
Alkaloid	Dragendroff's test	+
	Hager's reagent	+
	Mayer's test	+
	Wagner's test	+
Amino acid	Ninhydrin test	-
	Test for Cystine	-
	Test for Tyrosine	-
Protein	Biuret test	-
	Millons test	-
	Protein containing sulphur	-
	Xanthoproteic	-
Carbohydrate	Molish test	+
	Test for pentose sugar	+
	Test for reducing sugar	+
	Benedict's test	+
	Fehling's test	+
Flavonoid	Shinoda test	+
	Lead acetate test	+
	Zinc hydrochloride test	+
	Ferric chloride test	+
Phenol	Lead acetate test	+
Saponin	Foam test	+
Cardiac glycosides	Liebermann's test	+
Anthraquinone	Borntrager's test	+
Glycosides	Modified Borntrager's test	+
Triterpenoids	Noller's test	+

Quantitative standards

The efficiency and quality of the crude drugs were tested and the results were described in Table 2 and Table 3.

Table 2: Ash value

S. No.	Ash value	% w/w
1	Total ash	9.66%
2	Acid insoluble ash	5.02%
3	Water soluble ash	4.93%

Table 3: Extractive value

Sl. No	Extractive value	% w/w
1	Water soluble extractive value	8.72%
2	Alcohol soluble extractive value	7.41%

Loss on drying

The loss on drying of *Smilax zeylanica* was found to be 9.37%.

Total phenolic contents

The amount of phenolic content in *Smilax zeylanica* L. leaf extract, was determined by the Folin-Ciocalteu reagent, and expressed as the gallic acid equivalent (GAE) given in Table 4 and Fig. 5 and was found to be 0.1mg/ g. The findings implied that the phenolic components were responsible for the higher activity of antioxidant. Because of its ability to delay the oxidative breakdown of lipids and simultaneously enhancing the nutritional value and quality of food, phenol had gained popularity in the food business.

Table 4: Absorbance for sample and test compound

Concentration (mg/ ml)	Absorbance reading (Mean ±SEM)
20	0.220±0.026
40	0.306±0.029
60	0.431±0.029
80	0.671±0.020
100	0.793±0.015
Sample	0.036±0.003

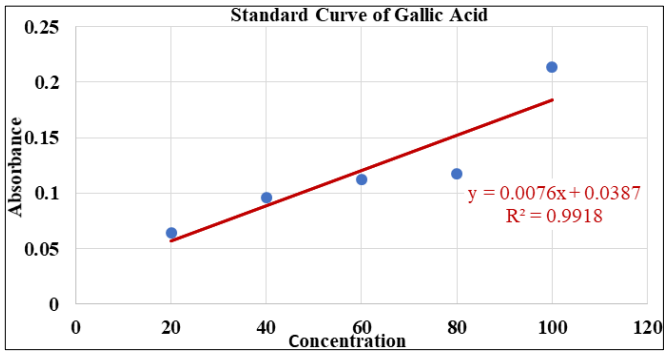

Fig 5: Standard Curve of Gallic Acid

Total flavonoid contents

The amount of flavonoid content in *Smilax zeylanica* L. leaf extract, was determined by the Folin-Ciocalteu reagent, and expressed as the gallic acid equivalent (GAE) given in Table 5 and Fig. 6 and was found to be 0.93 mg/ g. The significant antioxidant activity of flavonoids and their positive benefits on human nutrition and health had long been known. From the analysis it was found that significant amounts of flavonoids were present in *Smilax zeylanica*, extract which might have potent antioxidant activity.

Table 5: Observation of sample and test compound

Concentration (mg ml-1)	Absorbance (Mean ± SEM)
20	0.063±0.0009
40	0.095±0.0004
60	0.123±0.0225
80	0.117±0.0005
100	0.213±0.0001
Sample	0.056±0.002

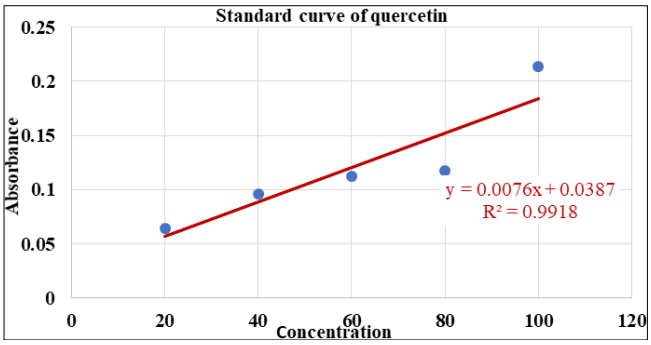

Fig 6: Standard curve of quercetin

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

The result of *in vitro* anti-inflammatory test described that ethanolic extract of *Smilax zeylanica* had protein denaturation of 31.34, 54.03, 78.65% at doses of 50, 100, 150 µg/mL respectively and for the standard drug (Acetyl salicyclic acid) it was found to be 27.11, 44.03 and 67.69% for same doses respectively as showed in Table 6. In Figs. 7 and 8, respectively *Smilax zeylanica* demonstrated good anti-

inflammatory action with a linear curve response. The inhibitory concentration of *Smilax zeylanica* was 154 and for the standard drug 139 which showed its high anti-inflammatory activity.

Table 6: Inhibition of albumin denaturation

Name of the sample	Concentration (µg/ml)	% stabilization (Mean ± SEM)	IC 50 (µg/ml)
Test (*Smilax zeylanica* extract)	50	31.34±0.0011	154
	100	54.03±0.0022	
	150	78.65±0.0017	
Standard	50	27.11±0.0015	139
	100	44.03±0.0012	
	150	67.69±0.0011	

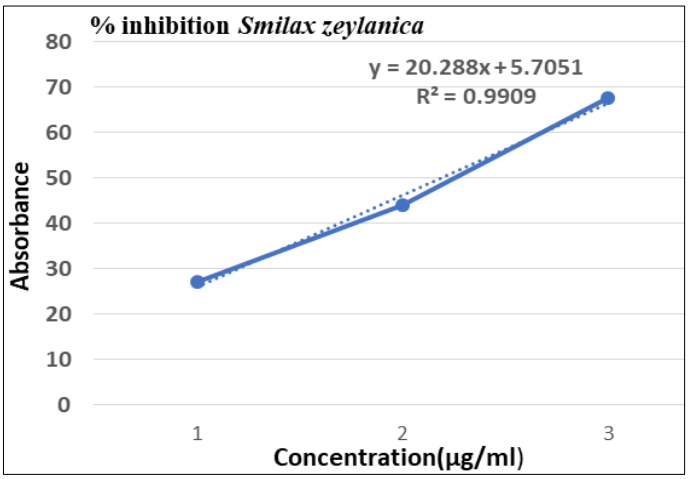

Fig 7: % inhibition *Smilax zeylanica*

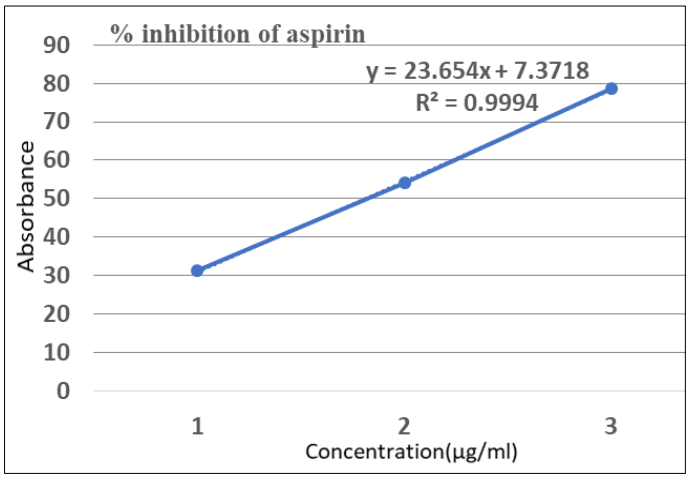

Fig 8: % inhibition *of* aspirin

***In vivo* anti-inflammatory activity**

Carrageenan-Induced Paw Edema

The result of *in vivo* anti-inflammatory test compared the efficacy of two doses of ethanolic extract of *Smilax zeylanica* (250 mg/kg, 500 mg/kg) against the standard drug as showed in Table 7. Carrageenan injections into the sub plantar region of control animals resulted in the inflammation which grew gradually over time as seen in Fig. 9. The inflammatory response caused by carrageenan in mice was greatly reduced by the oral administration of both dosages of the ethanolic leaf extract of *Smilax zeylanica* in a dose-dependent manner.

Table 7: Paw Edema Method

S. No	Group	Volume of Edema				
		0 min	60 min	120 min	180 min	240 min
1	Group-I (Control)	0.25±0.03	0.34±0.05	0.46±0.10	0.52±0.09	0.70±0.05
2	Group-II (standard)	0.27±0.03	0.38±0.03	0.36 ±0.03	0.33±0.02	0.30±0.03
3	Group-III (250 mg/kg)	0.28±0.04	0.36±0.05	0.33 ±0.05	0.31±0.05	0.29±0.04
4	Group-IV (500 mg/kg)	0.26±0.04	0.37±0.05	0.34±0.05	0.32±0.05	0.30±0.04

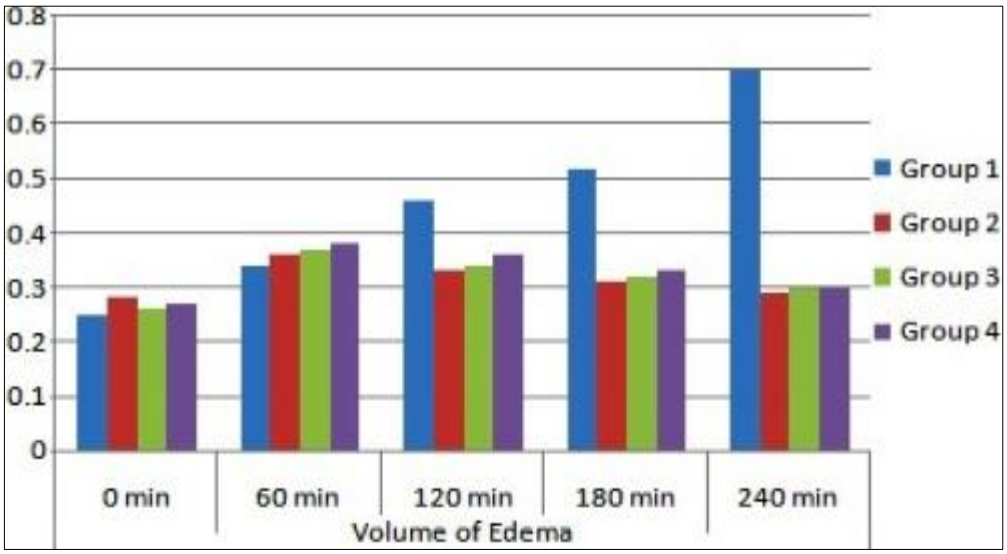

Fig 9: Percentage inhibition of inflammation of *Smilax zeylanica* extracts leaves using carrageenan induced mice paw edema.

Antimicrobial activity

The antimicrobial activity of *Smilax zeylanica* against *S. aureus* and *E. coli* with different concentration depicted in the Table 8. The least zones of inhibition was shown by the negative control and Gentamycin control exhibited the widest zones of inhibition against all the bacteria. The extract of *Smilax zeylanica* leaves showed increasing zones of inhibition. The activity of *Smilax zeylanica* leaves extract against *S. aureus* and *E. coli* was found to be higher at a concentration of 4 mg/disc followed by 2 mg/disc and 1 mg/disc respectively as shown in the Fig. 10. The maximum zone of inhibition was found to be 22 mm against *S. aureus* and 19 mm against *E. coli*. The leaves of Smilax zeylanica were shown to possess strong antibacterial properties.

Table 8: Antimicrobial susceptibility test of different fractions of *Smilax zeylanica*

S. No.	Microorganisms	Zone of inhibition (mm)				
		Ethanolic extract of *Smilax zeylanica*			Negative control	Gentamycin control
		1 mg/disc	2 mg/disc	4 mg/disc		
1	S. *aureus*	19	20	22	10	25
2	E. *coli*	15	18	19	12	20

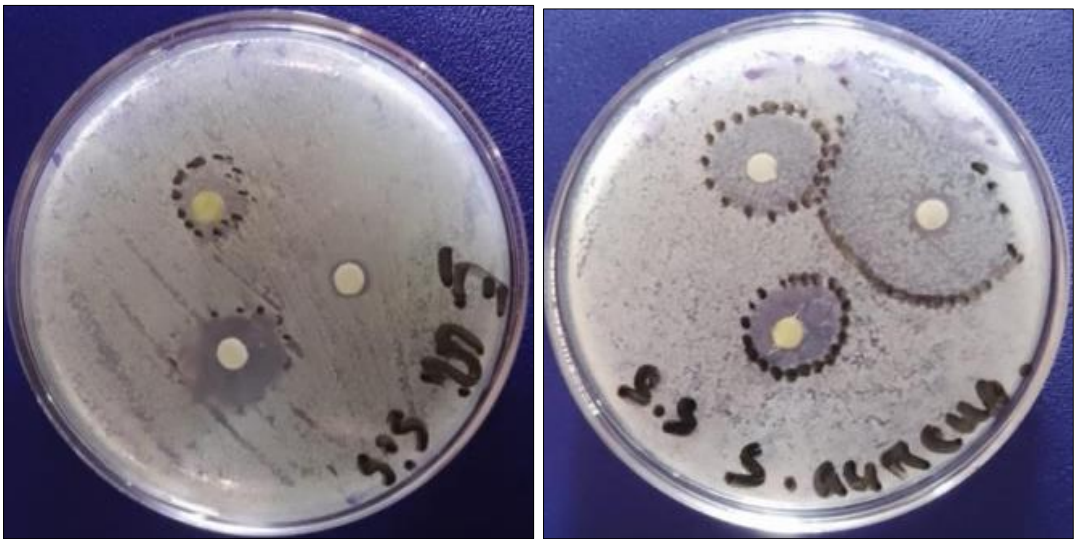

Fig 10: zone of inhibition of the sample against E. *coli* and S. *aureus*

Conclusion

The preliminary phytochemical screening of ethanolic extract of the leaves of *Smilax zeylanica* L. showed the presence of carbohydrates, tannin, flavonoid, phenol, terpenoids, cardiac glycoside, alkaloid, anthraquinones, and saponin.

This study suggested that the ethanolic extract of *Smilax zeylanica* L. leaves has antimicrobial properties. Also, it provides scientific understanding of the ethnomedical applications of *Smilax zeylanica* L., including the treatment of syphilis, gonorrhoea, and blood purification. *Smilax zeylanica* L. leaves have been screened for anti-inflammatory property which gave prominent positive result both *in vitro* and *in vivo* way which signifies that the leaf extract can be used for the treatment of inflammation. It also possessed antimicrobial activity against both gram (+ve) and gram (-ve) microorganisms and the results indicate that they contain compounds that may be used for antimicrobial and free radical scavenging purposes, making them viable sources of antioxidants for the pharmaceutical and animal feed industries.

Conflict of Interest

The authors declare that there is no conflict of interest.

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