

## Original Research Article

### Evaluation of in-vitro anti-inflammatory and antimicrobial properties of *Pergularia daemia* and *Solanum xanthocarpum*

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#### ABSTRACT

This work is of used on the evaluation of the anti-inflammatory and antimicrobial activities of stem and leaf extracts of *Pergularia daemia* and *Solanum xanthocarpum*. The prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of the anti inflammatory activity. The anti inflammatory activity of the ethanol extracts of stem and leaves were compared to that of the standard drug hydrocortisone. The percentage protection of lysis for Standard hydrocortisone 0.08 mg/ml is  $97.9 \pm 0.24\%$ , the maximum percentage protection of lysis was observed in ethanol extract of *Pergularia daemia* stem 12.0 mg/ml is  $78.58 \pm 0.33\%$ . *In vitro* antimicrobial activity of crude stem and leaf extracts of these 2 plants was tested by well diffusion method against 4 bacteria and 4 fungi. Among the two plant extracts tested, *Solanum xanthocarpum* showed more antibacterial activity against the organisms compared to *Pergularia daemia*. Therefore these herbal extracts could be used in future direction as alternative therapeutic agents for the treatment of human diseases.

#### Keywords

*Pergularia daemia*;  
*Solanum xanthocarpum*;  
in vitro anti-inflammatory;  
HRBC.

#### Introduction

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. The lysosomal enzymes released during inflammation produced a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal

constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane (Vadivu and Lakshmi, 2008). HRBC or erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization implies that the extract may as well stabilize lysosomal membranes. Human red blood corpuscle (HRBC) or erythrocyte

membranes are similar to lysosomal membrane components.

The most commonly used drug for management of inflammatory conditions are nonsteroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers (Tripathi, 2008; Bennett and Brown, 2005). For this reason, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is lack of scientific evidence.

*Pergularia daemia* Forsk ( Asclepiadaceae ) is a perennial twining herb known as Uttamarani in Sanskrit and Veliparutti in Tamil . The plant is useful in the diseases of vatha, convulsion, asthma, poisoning; the root is useful in mental disorder, anaemia, leprosy and piles (Sureshkumar and Mishra, 2007). Plant possesses stomachic, laxative and diuretic properties, useful in cough, biliousness and sore eyes. Leaf paste mixed with castor oil is applied to joints in inflammation, liver complaints, spleen enlargement; leaves have hypoglycemic activity (Sathish, et al., 1998).

*Solanum xanthocarpum* (Solanaceae) is a very prickly diffuse bright green perennial herb. It is one of the important medicinal plants distributed throughout India. The plants are useful as cough expectorant, cough, asthma, pain in chest, sore throat. The fruit juices of berries uses for treatment of sore throat. Stem, flowers and fruits are bitter, carminative, diuretic and dropsy. The leaves are used for piles. Juice of the leaves is given with black pepper in rheumatism. The leaves also applied locally, to relieve pain, a decoction of the plant is used in cases of gonorrhoea (Manandhar, 1986) Taking into consideration the medicinal value and utility, the present study has been initiated to

evaluate the *in vitro* anti-inflammatory and antimicrobial studies of *Pergularia daemia* and *Solanum xanthocarpum*.

## **Materials and Methods**

### **Preparation of stock solution of plant extract**

The plant parts, stem and leaf of *Pergularia daemia* and *Solanum xanthocarpum* were collected from 3-5 months old mature plants growing in the Garden of Mohamed Sathak College of Arts and Science, Chennai, Tamil Nadu, India and washed with sterile water and then chopped into small fragments. The materials were then shade dried at ambient temperature (32°C) for 10 to 15 days and the drying operation was carried out under controlled conditions to avoid chemical changes. The dried samples were crushed into fine powder using an electronic blender. The fine powder of the stem and leaf was extracted at 47°C by using soxhlet apparatus using ethanol as a solvent. After extraction, the extracts were dried at 50°C in hot air oven for 2 hours and they were stored properly for further studies.

### **In-vitro anti-inflammatory activity**

The human red blood cell (HRBC) membrane stabilization method (Gandhisan, et al., 1991) was used for this study. The blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Drug was prepared by 4 g of root, leaves and cultured cells were macerated with 10ml of hyposaline (0.36% NaCl) and extracts is centrifuged at 3000

rpm. Various concentrations of drugs were prepared (3.0, 7.0 and 12.0 mg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. Test solution was incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm (Vadivu and Lakshmi, 2008). Hydrocortisone was used as reference standard and a control was prepared omitting the extracts.

### **In vitro antimicrobial activity**

A total of four bacterial cultures (*Salmonella typhi*, *Vibrio cholerae*, *Staphylococcus saprophyticus* and *Escherchia coli*) and four fungal cultures (*Candida albicans*, *Penicillium* sp, *Fusarium* sp and *Aspergillus niger*) were used in this study. The cultures were procured from PG and Research Department of Biotechnology, Mohamed Sathak College of Arts and Science, Chennai, India. The bacterial strains were grown in nutrient broth at 37°C and they were stored on nutrient agar slants for future use. The fungal strains were grown in potato dextrose broth at 27°C and they were stored on potato dextrose agar slants for future use.

Anti-microbial activity of plant extracts was tested by a modified well-in agar method (Dhingra and Sinclair, 1995). From the nutrient broth and potato dextrose broth, the inoculum suspension was swabbed uniformly over the Muller Hilton Agar for bacteria and potato dextrose agar for fungi by using of sterile cotton swab. Subsequently, using a sterile borer, well of 0.5 cm diameter was made in the pathogen inoculated media. Different concentrations, i.e., 20, 40, 60 and 80 µl of each extract were aseptically filled into the well. Later the plates were placed at room temperature

for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 h at 37°C. The results were recorded by measuring the diameter of inhibition zone at the end of 24 h for bacteria and 48-72 hrs for fungi.

## **Results and Discussion**

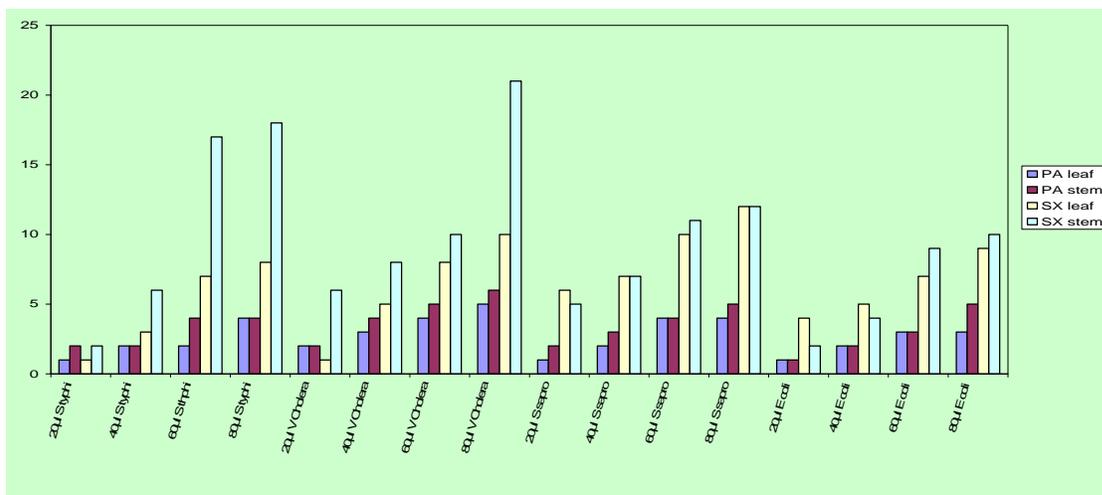
### **In-vitro anti-inflammatory activity**

The ethanol extracts of the stem and leaves of *Pergularia daemia* and *Solanum xanthocarpum* were studied for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. Among all the extracts showed significant anti-inflammatory activity in a concentration dependent manner. In both plants, Leaf extract at a concentration of 12.0 mg/ml showed maximum protection of HRBC (78.58±0.33% and 73.66±1.45%, respectively) in hypotonic solution and minimum protection was observed in stem extracts at 3.0 mg/ml (63.00±2.51% and 59.00±0.57% respectively). All the results were compared with standard hydrocortisone which showed above 92% protection (Table 1). The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane (Chou, 1997) and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release (Murugasan, 1981). The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components (Iwueke, 2006).

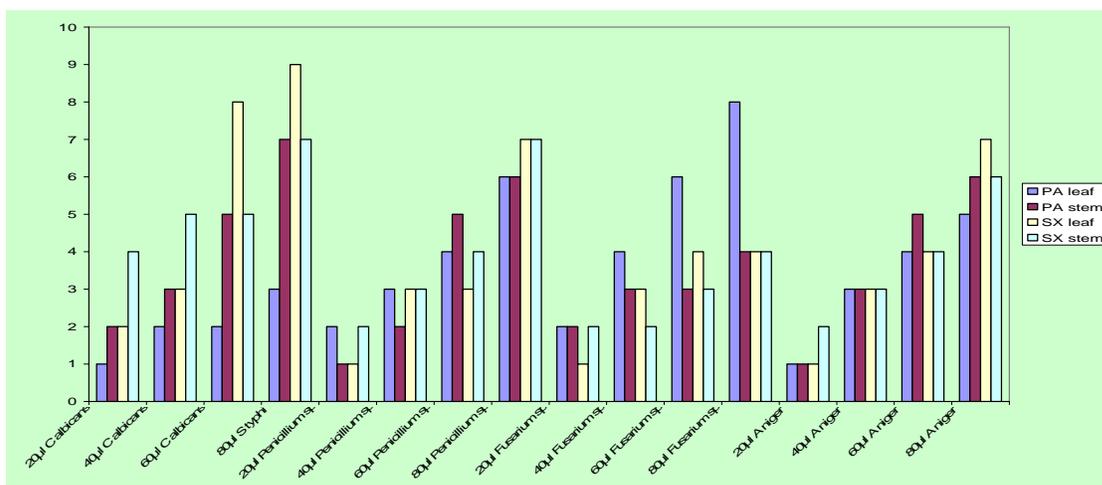
**Table. 1** In vitro Anti-inflammatory activity of *Pergularia daemia* and *Solanum xanthocarpum*

Con. Mg/L	Activity (Prevention of lysis %)				
	Hydrocortisone	<i>P. daemia</i>		<i>S. xanthocarpum</i>	
		Stem	Leaf	Stem	Leaf
0.03	92.8±0.07				
0.06	94.7±0.16				
0.08	97.9±0.24				
3.00		63.00±2.51	71.66±0.33	59.00±0.57	69.40±0.13
7.00		66.58±1.34	72.66±0.66	63.12±0.01	71.66±0.33
12.0		70.23±0.00	78.33±0.33	67.66±3.84	73.66±1.45

**Fig. 1** Anti bacterial activity of *Pergularia daemia* and *Solanum xanthocarpum*



**Fig. 2.** Anti fungal activity of *Pergularia daemia* and *Solanum xanthocarpum*



### Antimicrobial activity

The data pertaining to the antimicrobial potential of the plants extracts of *Pergularia daemia* and *Solanum xanthocarpum* are presented in Fig 1 and 2. Generally, all the bacteria and fungi under investigation were susceptible to all plant extracts caused significant growth inhibition of the test strains. *Solanum xanthocarpum* showed more antibacterial activity against the organisms compared to *Pergularia daemia*. Among the four bacteria and fungi tested against plant extracts, the maximum inhibition was observed in stem extracts of *Solanum xanthocarpum* against *Vibrio cholerae* and *Candida albicans* (21.0 and 9.0 mm respectively).

This variation in the effectiveness of the different extracts against different microorganisms depends upon the chemical composition of the extracts and membrane permeability of the microbes for the chemicals and their metabolism. It has been suggested that the antimicrobial activity is mainly due to the presence of essential oils, flavanoids and terpenoids and other natural polyphenolic compounds or free hydroxyl groups (Rojas et al., 2003).

In conclusion, the present investigation demonstrated that extracts from two plants, *Pergularia daemia* and *Solanum xanthocarpum*, possessed strong anti-inflammatory activity and antimicrobial activity. The potential anti-inflammatory and antimicrobial effects of these plants could be enhanced by extracting with ethanol instead of water as applied in the traditional practice.

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