

# EVALUATION OF HEAT STABLE PROTEINS, TOTAL SOLUBLE PROTEINS AND ANTIBACTERIAL PROPERTIES OF *COSTUS PICTUS* D. DON

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## KEY WORDS

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## ABSTRACT

*Costus pictus* D. Don or *Costus mexicanus* belongs to family costaceae. People of Karnataka and Kerala use the leaves of this plant to treat diabetes. It has hypoglycemic and antihelmenthic properties and the plant are rich in antioxidants. Heat stable proteins and total soluble proteins analysis revealed high HSP and TSP content on the leaves. Antimicrobial activity of the plant was evaluated against human pathogenic bacteria viz., *Escherichia coli*, *Pseudomonas*, *Bacillus subtilis* and *Staphylococcus aureus*. The HSP of the leaves of the plant used for the study revealed significant growth inhibitory effects. The results showed that the minimum inhibitory concentration of the leaf protein extract against *Staphylococcus aureus* and *Pseudomonas* was at 50 $\mu$ L/mL. MIC of *Bacillus subtilis* and *Escherichia coli* was at 75 $\mu$ L/mL.

## INTRODUCTION

In developing countries like India, contagious diseases caused by microorganisms account for maximum health problems. The indiscriminate use of allopathic antimicrobial drugs has resulted in microbial resistance to these drugs. Hence researchers are keen on biologically active compounds isolated from plants for the elimination of pathogenic microorganisms because of the resistance that they have developed to antibiotics (Hunter and Reeves, 2002). The continuous evolution of bacterial resistance to antibiotics has necessitated the search for novel and effective antimicrobial compounds (Fagbemi *et al.*, 2009).

The plants are being used from ancient times to cure diseases. Medicinal plants are immensely contributing to the primary health care of human beings from the time immemorial. Medicinal plants are natural composite sources that act as new anti-infectious agents (Ushimaru *et al.* 2007). They are used against many ailments which includes infectious diseases caused by bacteria. *Costus pictus* is an important medicinal plant and the leaf of which is used by people of Karnataka and Kerala to treat diabetes. It is also called insulin plant, spiral ginger and step ladder. Its antidiabetic property is reported by Merina (2005) and Nandakumar *et al.*, (2007). The plant is also rich in antioxidants (Shubha and Anusuya, 2010). As there is no report on the antibacterial activity of the plant, the present work was undertaken to study the antibacterial properties of the leaves against human pathogenic bacteria.

## MATERIALS AND METHODS

### The plant material

The medicinal plant *Costus pictus* was brought from western ghat and grown in the herbal garden of Maharani's Science College for Women, Bangalore, Karnataka, India. Leaves from well grown and healthy plants were used for the study.

### HSP and TSP analysis

The leaves were collected and washed with distilled water several times and kept over filter paper for drying. 10g of leaves were placed in mortar and pestle and blended with prechilled acetone. The slurry obtained is then filtered through Whatmann filter paper by adding chilled acetone over the funnel. The extract is air dried and is stored in sealed condition at - 40°C until use.

### Extraction of total soluble proteins

1g of acetone extract was stirred with extraction buffer containing Tris-EDTA and thiol compounds and precipitated with 10% TCA. The slurry was centrifuged at 15,000 rpm for 20 min at 4°C. The supernatant was taken and volume was measured. The TSP was quantified at 280nm and aliquot was kept in the refrigerator.

### Extraction of heat stable protein

TSP was incubated at 70°C for 10 minutes and then centrifuged at 12,000 rpm for 20min at 4°C to remove the precipitated heat labile protein. The protein content was determined by Lowry's method (Lowry *et al.*, 1951).

### Bacterial cultures

*Escherichia coli*, *Pseudomonas*, *Bacillus subtilis* and *Staphylococcus aureus* (ATCC type) were procured from

**Table 1: Zone of inhibition (mm)**

Extract( $\mu\text{L/L}$ )	<i>Escherichia coli</i>	<i>Pseudomonas</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	Tris-EDTA
25	0	0	0	0.07 $\pm$ 0.01	0
50	0	0.9 $\pm$ 0.11	0.8 $\pm$ 0.23	0.95 $\pm$ 0.11	0
75	0.9 $\pm$ 0.13	13 $\pm$ 0.23	12 $\pm$ 0.37	10 $\pm$ 0.37	0
100	12 $\pm$ 0.2	14 $\pm$ 0.42	13 $\pm$ 0.22	12 $\pm$ 0.36	0

p < 0.05

Victoria Hospital, Bangalore and maintained on nutrient agar medium.

#### Antibacterial activity

The HSP was used for the study. The bacteria were grown in the Mueller Hinton agar media at 37°C and maintained at 4°C. Antibacterial assay was carried out by a modified agar well diffusion method (Perez *et al.*, 1990). The sterile media containing agar was poured into the sterilized Petri plates and allowed it to solidify at room temperature. 1000 $\mu\text{L}$  of bacterial suspension was spread on the solidified medium using sterile glass spreader. Wells were made in the medium using cork borer. Different volume of the extract was poured into the well and incubated for 24hrs at 37°C. Tris-EDTA was used as control. The experiment was carried out in triplicates and data was analyzed statistically.

#### RESULTS AND DISCUSSION

The leaf sample contained 0.9mg/mL HSP and 7.8mg/mL TSP. Antibiotics provide the main basis for treating infectious diseases. Hence, there is an increase in the investigations on plants as a source of human disease management (Prashanth *et al.*, 2001 and Woldemichael *et al.*, 2003). Medicinal plants are very good sources of antimicrobial agents (Mahesh and Satish, 2008). An extensive research has been done on the effects of plant extracts on bacteria (Reddy *et al.*, 2001 and Ateb and Erdourul, 2003). Table 1 shows that the leaf extract of *Costus pictus* was effective against all the bacteria studied with inhibition zone ranging from 0.07mm to 14 mm and with a concentration of 25 to 100  $\mu\text{L/L}$ . For *Pseudomonas*, and *Bacillus subtilis* at 50  $\mu\text{L/L}$  the zone of inhibition was 0.3mm and 0.8mm. The Table 1 shows that the minimum inhibition concentration for *Escherichia coli* was 75  $\mu\text{L}$  with a zone of inhibition of 0.9mm diameter. At 100  $\mu\text{L/L}$  of leaf protein extract zone of inhibition was maximum for all the bacteria tested. As the concentration of the extract increased, the diameter of inhibitory zone was also increased. The results proved that the leaf protein extract of *Costus pictus* is having significant antimicrobial activity. *Gymnema sylvestre*, another widely used antidiabetic plant showed similar antimicrobial activity against four bacteria (Satdive *et al.*, 2003). Hence the use of *Costus pictus* leaf not only protects the diabetic patients from pathogenic bacteria but also nourishes the diabetic patients with proteins which also serve as antimicrobial. But further phytochemical evaluation is essential for the antimicrobial

effect of this medicinal plant.

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