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SCREENING OF THE INFLORESCENCE OF *COSTUS SPECIOSUS* (J. E. SMITH) FOR VARIOUS BIOLOGICAL ACTIVITIES

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ABSTRACT

Costus speciosus is one of the most traditionally used plants for its various pharmacological activities it has been used as anti-diabetic as anthelminthic as contraceptives and aborting agents. Pharmacognostic and phytochemical studies have been conducted on the stems leaves roots and rhizomes but the inflorescence have not been much studied. The current study deals with the screening of the ethanolic extract of *Costus speciosus* inflorescence for anti-oxidant and anti-bacterial activity using inhibition zone comparision to Ciprofloxacin.

Keywords: *Costus speciosus*, anti-bacterial, Ciprofloxacin, Antioxidant.

INTRODUCTION

Medicinal plants have been of great importance to the health care needs of individuals and their communities. The use of herbal preparations made from medicinal plants is widespread in developing countries. There is a growing demand for natural products of medicinal/pharmaceutical importance in both domestic and international market. The current demand for the plant based products in medicine and industry has resulted in extensive investigation of the plants for potential therapeutic agents. Many traditional practitioners across the world have valuable information of many plants for treating wounds and burns. The presence of bioactive constituents in plants has urged researchers to screen medicinal plants with a view to determine potential wound healing activities and isolate chemical entities associated with wound healing. Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. The large consumption of antibiotics in an intensive care to protect patients from inflammations of disease after surgery, has led to the emergence of bacterial resistance to important bacteria species such as Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumonia. Consequently, it becomes necessary to use alternative natural resources like medicinal plants, which are safe for human health, and reduce the risk of treatment by antibiotics. The plant Costus speciosus (Costaceae) has been reported for its various biological activities. In the present study the inflorescence of Costus speciosus have been used based on the study conducted on other parts of the plant in literatures [1-4].

MATERIALS AND METHODS

Materials

Chemicals and reagents used Ethanol, Mayer's reagent, Dragendroff's reagent, Sulphuric acid (dilute and concentrated), Carbon tetrachloride, dilute ammonia, strong solution of lead acetate, Chloroform, glacial acetic acid, Ferric chloride (10% w/v), Magnesium powder, Hydrochloric acid (dilute and concentrated), Ruthenium red, Biuret reagent, Ninhydrin reagent, Absolute alcohol, Acacia gum, Hard paraffin, White soft paraffin, Wool fat, Cetostearyl alcohol, Glucose, Ascorbic Acid, di-Sodium hydrogen phosphate, Ammonium Molybdate (Tetrahydrate), Monobasic potassium phosphate, Dibasic potassium phosphate, Sodium Potassium Tartrate, 3,5-dinitrosalicylic acid, Amylase, Starch soluble, Distilled water, Hydroxyproline, ALT kit, AST kit, Glucose (GOD-PAP) kit, Total Cholesterol kit, Sodium citrate, Diethyl Ether, Albumin, Xylene, Hematoxylin, Eosin.

All the chemicals were purchased from Nice chemicals (Cochin), Rajesh chemicals (Mumbai), Ozone international (Mumbai).Drugs used Glibenclamide, Phenobarbitone, Metronidazole, Gentamicin disc Ciprofloxacin disc.

Apparatus used

Elastic bandages, Tapes, Sterile forceps, surgical blades, biopsy punch, fluoride tubes, standard flasks, beakers, motor pestle, spatula, Funnel, Filter paper, 26.5 G needle, 1.0 ml syringe, 5.0 ml syringe, Filter paper discs, Petri dish, Conical flaks, inoculating loop.

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Instruments used

Electronic balance, heating mantle, water bath, Rotary vacuum evaporator, Semi-automatic chemical analyzer, UV-Visible Spectrophotometer, Colorimeter, Magnetic stirrer, pH meter, incubator, Autoclave.

Animals

Female Albino Wistar rats of 150-250g and Rabbits were used for the experiment. Animals were allowed to be acclimatized for a period of one week in the laboratory prior to the study. The animals were housed under standard laboratory conditions (i. e. 12-hour light and dark sequence; at an ambient temperature of 25 ± 2°C, 35-60% humidity); the animals were fed with standard diet and water ad libitum.

The use and care of the animals in the experimental protocol has been approved by the Institutional Animal Ethical Committee (IAEC) (Regd. No: PCP/2016/IAEC//) as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Test organism:

Escherichia coli, Staphylococcus aureus cultures were selected for the study from the samples in Pushpagiri College of Pharmacy.

METHODS

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY OF THE EXTRACT [5-8]

Ammonium Molybdate Reduction Method

Reagent solution preparation

50 ml reagent solution was prepared by dissolving 198.7 mg Sodium Hydrogen Phosphate (Mol. Wt. 141.96 g/mol), 247.17 mg Ammonium Molybdate Tetrahydrate, 0.16 ml of Sulphuric acid (97% purity) and making the volume with distilled water.

Extract of a particular concentration preparation

1 mg/ml, concentrations of the extract was prepared in distilled water.

Ascorbic Acid of a definite concentration preparation

50, 100, 150, 200, 250, 300, 350 µg/ml ascorbic acid solutions were prepared and calibration curve plotted.

Method

0.3 ml of the extract and different concentrations of Ascorbic acid was taken in different test tubes and 3.0 ml of reagent was added to each of them and incubated for 90 minutes at 95°C after cooling to room temperature absorbance was measured at 695nm using digital colorimeter. 0.3 of distilled water was taken in case of blank. The antioxidant capacity was expressed as number of equivalents of Ascorbic acid. The experiments were performed in triplicate and mean value was used. The equation used was

Y = b0X + b1, where Y is the absorbance X is the concentration and n is the number of concentration of ascorbic acid used

$$b0 = \frac{n \sum XY - \sum X \times \sum Y}{n \sum X2 - \sum X2}$$

$$b1 = \frac{\sum Y - b0 \sum X}{n}$$

EVALUATION OF ANTI-BACTERIAL ACTIVITY OF THE ETHANOLIC EXTRACTS OF INFLORESCENCE OF COSTUS SPECIOSUS J. E. Smith. [9-13]

Cultures of bacteria were grown on nutrient broth at 4°C. The extracts were dissolved in ethylene glycol and filtered through membrane filter (0.47 m) and it's used for antibacterial activity using disc diffusion method. A concentration of 30µg / disc was chosen. Sterile 6 mm diameter filter paper disc were impregnated with 30µg of sample and placed on the sterile media spreaded with test bacteria. The plates were incubated at 37°C for 12 hours. The experiments were carried out in triplicate. The results were recorded by measuring the zone of growth inhibition around the disc. For comparison, standard antibiotic Ciprofloxacin was included in the assay.

STATISTICAL ANALYSIS [14]

Statistical data were expressed as mean +/- S.E.M and evaluated by one-way ANOVA followed by Student's t-test/Dunnett's multiple comparision test. The data were considered significant at $p < 0.05$. Statistical analysis was done using IBM SPSS version 22.0.

RESULTS

TOTAL ANTIOXIDANT CAPACITY OF THE ETHANOLIC EXTRACT OF THE INFLORESCENCE OF *COSTUS SPECIOSUS* J. E. Smith.

The total antioxidant capacity of the Ethanolic extract of the inflorescence of *Costus speciosus* J. E. Smith was found to be 0.16 mg Ascorbic acid equivalents/ 0.3 mg of extract.

EVALUATION OF ANTI-BACTERIAL ACTIVITY OF THE ETHANOLIC EXTRACTS OF INFLORESCENCE OF *COSTUS SPECIOSUS* J. E. Smith.

The Extract showed significant anti-bacterial activity compared to the standard Ciprofloxacin in terms of the inhibition zone.

Table 1. Plant material used

Sl. No.	Plant name	Part used	Family	Collection period	Place of collection
1	*Costus speciosus*	Inflorescence	Costaceae	October - November	Kaviyoor and Vallamkulam regions of Thiruvalla taluk

Table 2. Determination of Total Antioxidant capacity

Sl. No:	Concentration (µg/ml)	Absorbance*
1	50	0.156 ± 0.006
2	100	0.315 ± 0.006
3	150	0.465 ± 0.003
4	200	0.617 ± 0.006
5	250	0.777 ± 0.006
6	300	0.888 ± 0006

*Value are mean ± S. E. M, n=3

Table 3: Inhibition zone for Test and standard using *Escherichia coli and Staphylococcus aureus*

S. No	Bacterial Strains	Inhibition zone (mm)		Percentage of inhibition
		Standard Ciprofloxacin 10 µg/Disc	Test Extract 30µg/Disc	
I	**Gram positive organism**	11.33 ± 0.33*	6.33 ± 0.33*	54.66 ± 3.24*
1.	*Staphylococcus aureus*			
II	**Gram negative organism**	12.33 ± 0.33*	7.67 ± 0.33*	66.25 ± 2.05*
1.	*Escherichia coli*			

Data represents Mean ± S.E.M. (n=3). *p< 0.05 Significant compared to Standard

Figure 1. Calibration curve of Ascorbic acid for the determination of Total antioxidant capacity.

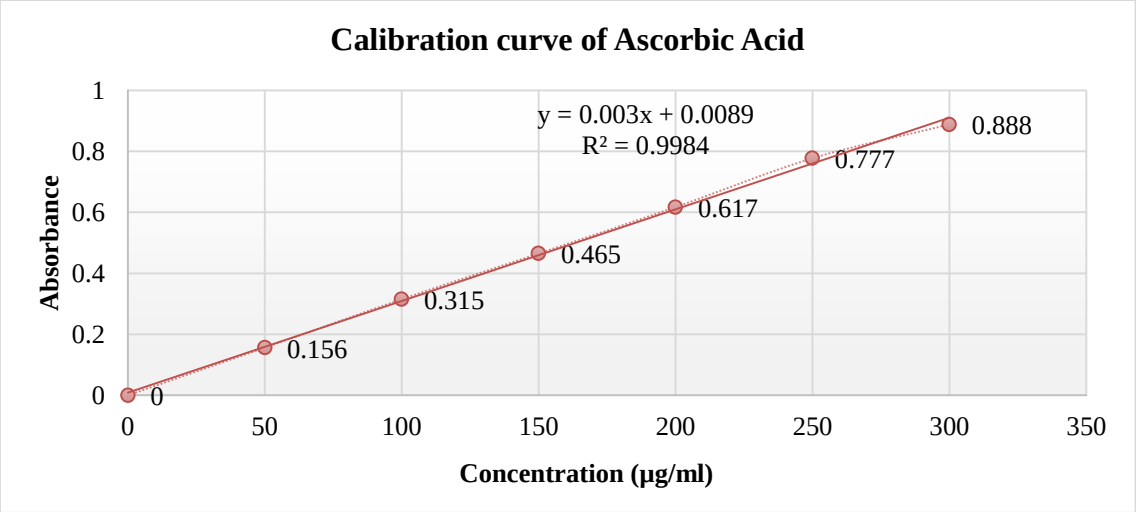


Figure 2. Inhibition zones for *S.aureus.*

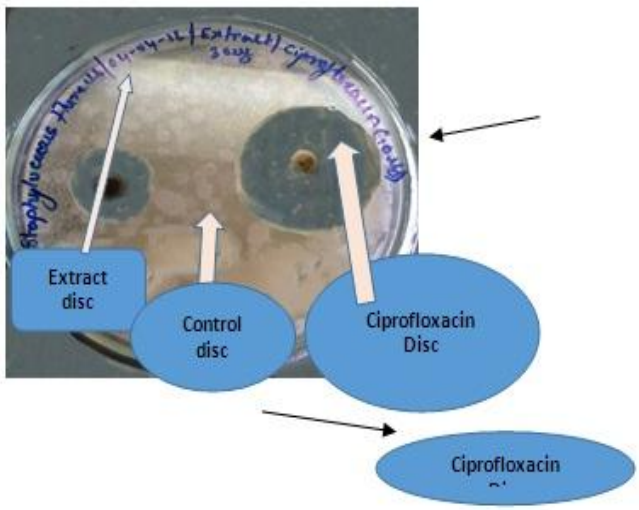
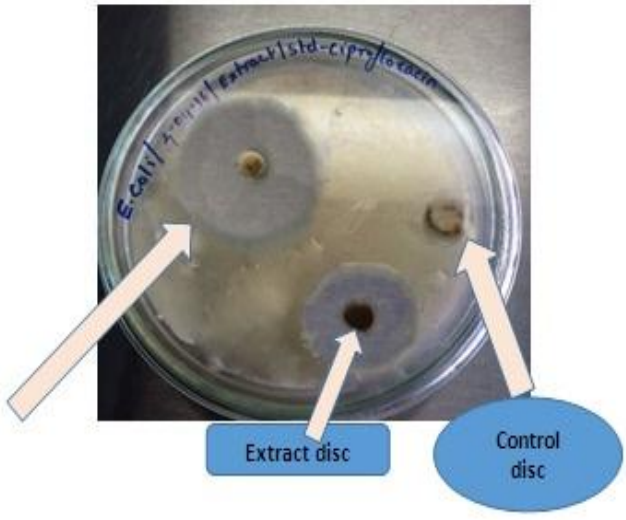


Figure 3. Inhibition zones for *E.coli.*



DISCUSSION

Total Antioxidant capacity

The total antioxidant capacity assay is a spectrophotometric method for the quantitative determination of Antioxidant capacity, through the formation of Phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) and subsequent formation of green phosphate / Mo (V) complex at acidic pH. It evaluates both water soluble and fat soluble anti-oxidants. The antioxidant potential of the extract was markedly increased with the increase in the concentration.

Antibacterial study

Significant percent inhibition was seen for the extract when compared to ciprofloxacin indicating the antibacterial effect of these extract thus this may also be one of the mechanism assisting in wound healing. Ciprofloxacin is a broad-spectrum antibiotic of the fluoroquinolone class. It is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, and a type II topoisomerase, topoisomerase IV, necessary to separate bacterial DNA, thereby inhibiting cell division. The extract showed significant activity against both Gram-positive and Gram-negative bacteria but less than the standard.

CONCLUSION

However, still many herbal folk medicines for diabetes, wound healing and infections have not undergone thorough scientific investigation and careful assessment of their effects. Hence it is the need of time to consider all such folk use based herbal medicines for determining their pharmacological activities and isolating single drug entity responsible for antioxidant and anti-bacterial activities. In conclusion the present study revealed the antioxidant and antibacterial activity of the inflorescence of *Costus speciosus*.

All these scientific observations support the above fact. Further study is required on tissue cultures of this plant and the isolation of compounds responsible for these pharmacological activities. These investigations may pave a way for a better drug development.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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