Ancient Science of Life, Vol No. XIV 4 April 1995 Pages 286-291

ANTIMICROBIAL PROPERTIES OF *IXORA COCCINEA* L. (RUBIACEAE)

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Received: 17 November, 1994 **Accepted: 25 January, 1995**

ABTRACT: *Antimicrobial activity was detected in the 50% ethanolic extract of Ixora coccinea. The effective inhibitory concentration of the extract against both the bacterial test organisms and the fungal test organisms studied was 125 ug mL^{-1}, beyond which the inhibitory activity declined and the organisms started reviving from the effect of the antimicrobial principle.*

INTRODUCTION

A large number of Indian medicinal plants are regularly employed as antibiotic agents by practitioners of Ayurveda and Unani system of medicine. In fact, the use of plant material in chemotherapy by the rural population far exceeds the total employment of modern medicaments in the country (1).

Ixora coccinea L. is a shrub growing throughout India. It is extensively used in traditional medicine. The flowers are used to cure dysentery, leucorrhoea and bronchitis. A decoction of the leaves is employed as a lotion for eye troubles and can cure sores and ulcers. The roots possess astringent and antiseptic properties (2,3).

A perusal of literature revealed that *I.coccinea* flowers had not been subjected to screening for antimicrobial properties. In this paper, we report the presence of antimicrobial principles in the flowers of *I.coccinea.*

MATERIALS AND METHODS

Preparation of the extract of flowers of *I.coccinea*

The flowers were collected locally, the shade – dried plant material was finely powdered and extracted with 50% ethanol. The filtered extract was concentrated under reduced pressure and diluted with sterile normal saline to required concentrations and used for antimicrobial studies.

Microbial strains and culture media

The bacterial strains used for the assay of the antimicrobial principle from *I.coccinea* were *Escherichia coli* and *Staphylococcus aureus.* The fungal strain used was *Aspergillus niger.* These strains were obtained from the Department of Microbiology, Medical College, Thiruvanthapuarm. The culture media used were Nutrient agar for bacterial and Sabouraud's agar for the fungus.

Assay of antimicrobial activity

A paper disc methods was employed for the preliminary assay. Petri plates containing nutrient agar were seeded with one-day-old cultures of *E.coli* and *S.aureus.* Sterile paper discs (6 mm diameter) containing 25 µg and 250 µg of the plant extract were placed on the surface of the medium. Filter paper discs with normal saline served as the control. Standard antibiotic disc containing 25 µg of chloramphenicol served as the reference. Antimicrobial activity was measured as the diameter of the clear zones developed around the paper discs.

Determination of the effective inhibitory concentration against bacteria

Gram +ve bacteria *S.aureus* and gram –ve bacteria *E.coli* were allowed to grow in culture tubes containing nutrient broth. Different concentration of the plant extract, 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250 µg were added to the cultures. The bacterial inoculum contained 10^5 cells $mL^{-1.}$ The inoculated tubes were incubated at 37^0C for 18h. Standard antibiotic chloramphenicol served as the reference. The turbidity caused by the bacterial growth was estimated using a spectrophotometer at 420 nm.

Against Fungus

A.niger was the test organism used. The tests conducted were a) spore germination study b) mycelial growth.

a) Spore Germination study

A drop of the spore suspension (10^3 conidia mL^{-1}) was placed on the sterilized grooved slides along with one drop of the fungal culture broth. Different concentrations of the plant extract were also added to these slides and incubated at 30^0C. Slides without the extract served as control. Germinated spores on the slides were counted after 6h, when spore germination started, up to 9h at an interval of 30 min using as inverted microscope.

b) Mycelial growth

To each flask containing 10ml Sabouraud's broth, 0.1 ml of the spore suspension (10^3 conidia mL^{-1}) was added. Different concentrations of the plant extract were also added to the flasks and incubated at 30^0C in a shaker incubator. Flasks containing 25 µg of clotrimazole served as control. Mycelial growth was estimated as the dry weight of the mycelium after 48 hours incubation. The mycelial pellets were filtered on previously weighed filter paper and dried at 60^0C for 8 h to get the dry weight.

RESULTS AND DISCUSSION

Assay of antimicrobial activity

The antimicrobial principle of the plant extract inhibited the growth of both the bacterial test organisms employed in the study, as evidenced from the clear zones formed around the discs. Discs with 25 µg of the plant extract showed 6 mm clear zones for both the bacterial strains and discus with 250 µg gave 8 mm clear zones. However it was not as active as the reference antibiotic chloramphenicol which gave 3.0 cm clear zone for *E.coli* and 1.8 cm clear zone for *S.aureus.*

Determination of the effective inhibitory concentration Against bacteria

The effective inhibitory concentration of the antimicrobial principle against both the bacterial test organisms was 125 µg mL^{-1}. There was a steady increase in the growth

inhibitory activity of the antimicrobial principle up to a concentration of 125 µg mL^{-1} and thereafter it declined. The organisms revived and developed a resistance to the antimicrobial principle beyond 125 µg mL^{-1}. (Table 1).

Against fungus

The results of fungal spore germination studies showed the plant extract inhibited spore germination. However it is not as active as the reference antibiotic used (clotrimazole).

The mycelial growth measured as the dry weight also showed steady decrease up to a concentration of 25 µg mL^{-1} of the antimicrobial principle, beyond which the degree of inhibition of mycelial growth declined. The effective concentration for both the inhibition of spore germination and mycelial growth was 25 µg mL^{-1} (Table 2).

The antimicrobial activity of plant extracts is already established (1,4,5). The results of our experiment clearly indicate that the extract of *I. coccinea* flowers is active against gram +ve and gram –ve bacteria and also fungal organisms. The antimicrobial principle is active only at lower concentrations and beyond a concentration of 125 µg mL^{-1}, the activity decreased and the organisms revived from the effect of the antimicrobial principle. This may be due to the activation of the resistant mechanism in the microbial cells are higher concentrations of the antimicrobial principle. As the plant extract we used is the crude one, it may perhaps contain some components which help the microbes overcome the inhibitory effect and this is made available in sufficient quantity at higher concentrations. Hence the decline in growth inhibitory activity of the organisms beyond the effective concentrations (125 µg mL^{-1}).

Previous phytochemical analysis of the plant (6,7) have shown the presence of biologically active constituents such as rutin, a yellow colouring matter, quercetin, and leucocyanidin glycoside. These compounds may have been responsible for the antimicrobial activity observed in the present study.

ACKNOWLEDGEMENT

We thank Dr. P. Pushpanganda, the Director of the Institute of facilities.

TABLE -1

Effect of *Ixora coccinea* flower extract on bacterial growth.

Concentration of extract (µg / ml)	***E. coli*** **(OD value)**	***S. aureus*** **(OD value)**
0 (Control)	0.705	0.808
25	0.527	0.553
50	0.361	0.583
75	0.270	0.605
100	0.257	0.552
125*	0.226	0.332
150	0.527	0.512
175	0.596	0.605
200	0.609	0.643
225	0.688	0.662
250	0.617	0.586
Chloarmphenicol (Std. disc 25µg)	0.010	0.043

* Effective inhibitory concentration.

TABLE -2

Effect of *Ixora coccinea* flower extract on the germination of spores and mycelial growth of *Aspergillus niger* .

Concentration of extract (µg / ml)	Spore Germination (%)	Mycelial dry weight (mg)
0 (Control)	86.20	169
25	66.14	135
50	60.73	130
75	66.91	132
100	60.07	126
125*	41.34	123
150	61.87	149
175	70.07	146
200	73.26	142
225	70.14	158
250	80.12	170
Clotrimazole (Std. disc 25µg)	32.75	105

* Effective inhibitory concentration.

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