

## Research Article

### A COMPARATIVE ANTIBACTERIAL STUDY OF *IXORA COCCINEA* L. PLANTS WITH RED, ORANGE, PINK AND WHITE FLOWERS

SOWJANYA. PULIPATI\*, SUSHMA. P, V. JHANSI LAKSHMI, P. SRINIVASA BABU

*For Author affiliations see end of the text*

This paper is available online at [www.jprhc.in](http://www.jprhc.in)

---

#### ABSTRACT

Eradication of pathogenic bacterial infection is the most effective means of curing several diseases and preventing recurrent episodes. The main objective of this study was to evaluate the comparative antibacterial activity of *Ixora coccinea* L. plants with red, orange, pink and white flowers against tested bacteria *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*. Both the methanolic and aqueous extracts of all four types of flowers exhibited considerable antibacterial activities against bacteria. The methanolic extracts of red and pink flowers exhibited high antibacterial activity whereas orange flowers showed moderate activity while white flowers showed low activity. The aqueous extracts displayed less inhibitory effects compared to the methanolic extracts. The aqueous extracts of red and pink flowers showed considerable high antibacterial activity compared to orange and white flowers. The preliminary phytochemical screening revealed the presence of alkaloids, glycosides, steroids, carbohydrates, flavonoids and tannins.

**Key words:** *Ixora coccinea*, antibacterial activity, cup-plate diffusion method

#### INTRODUCTION

Medicinal plants are the bioresources gifted by nature used to cure various human diseases. To determine their potential as sources for new drugs, it is essential to study the medicinal plants [1]. In contrast to synthetic drugs, antimicrobials of plant origin are not associated with side effects and have enormous therapeutic potential to heal many infectious diseases. Hence natural products provide unlimited opportunities to develop a new drug. Therefore, researchers are increasingly turning their attention to folk medicine to develop better drugs with no side effects against microbial infections [2].

The genus *Ixora* contains more than 400 species and is one of the low-growing evergreen perennial shrub through Southeast Asia. *Ixora coccinea* is commonly known as 'Jungle-geranium' and 'Flame of the woods'. It is a popular flowering shrub belongs to the Rubiaceae family. The "wild" flower colour is red or red-orange, but ornamental varieties are white, yellow, pink flowers. A decoction of the roots is given for dysentery and as a sedative for hiccoughs, nausea and loss of appetite, fever and gonorrhoea. In the traditional medicine the leaves and roots are used to treat a wide variety of ailments like hepatoprotective, chemoprotective, antimicrobial, antioxidant, antinociceptive, anti – inflammatory, dysentery, ulcers & gonorrhoea [3]. Pharmacologically the leaves are reported for their antimicrobial, antidiarrhoeal and antinociceptive activities [4,5,6]. Flowers possess antioxidant, anti-inflammatory, wound healing activities [7,8,9].

A review of literature did not reveal any information on the antibacterial activity of flowers of *Ixora coccinea*. From this view point the present study was carried out to evaluate the antibacterial activity of *Ixora coccinea* L. plants bearing red, pink, orange and white flowers against tested bacteria.

## MATERIAL & METHODS

### Plant material

The flowers of *Ixora coccinea* L. were collected from different localities of Guntur District and authenticated by Mrs. M. Vijaya lakshmi, Department of Botany & Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh. The flowers were collected, shade dried and then powdered to get a coarse powder.

### Preparation of extract

The powdered material is extracted by hot percolation extraction using methanol and water in soxhlet extraction apparatus. The red, orange, pink and white flowers were extracted separately with methanol and water. The extracts were dried in a rotary vacuum evaporator.

### Phytochemical screening

The air-dried flowers of *I. coccinea* L. was reported for the presence of cycloartenol esters, lupeol fatty ester, lupeol, ursolic acid, oleanolic acid, and sitosterol [10]. The screening revealed the presence of alkaloids, glycosides, tannins, flavonoids, steroids and carbohydrates. These were identified by characteristic color changes using standard procedures [11, 12]. The presence of phytoconstituents was reported in (Table 1).

### Preparation of Inoculums

The cultures of Gram positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative *Escherichia coli*, *Klebsiella pneumoniae* were collected from Department of Microbiology, Acharya Nagarjuna University, Guntur. The stock cultures were maintained in nutrient agar medium. The suspension of all organisms i.e., *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsiella pneumoniae* were prepared by inoculating a loopful of the suspension in 20 mL of nutrient broth in conical flask and incubated in shaker incubator at 37°C for 24 hours to activate them.

### Antibacterial activity (Cup- plate diffusion method)

The extracts obtained from flowers of *Ixora coccinea* L. were studied for antibacterial activity. The antibacterial activity of methanol and aqueous extracts was assayed by using Cup-Plate diffusion method. To determine the susceptibility patterns of the organisms against extracts, the overnight grown cultures in nutrient broth served as inoculums. The petri dishes were sterilized and labeled with the name of the extracts and bacteria. The nutrient agar medium (15 mL) was sterilized in test tubes and inoculated with 100 µL of bacterial suspension at 40-45°C and poured into the corresponding labeled petri dishes. The cups of diameter 6mm were made equidistantly in the agar plate with a sterile borer. The methanol and aqueous extracts were dissolved in DMSO (Dimethyl sulphoxide) and a stock concentration of 500mg/ml was prepared. 25 µL and 50 µL samples were taken from 500mg/ml concentration with micropipette and added to the cups. 25 µL of DMSO was used as a control. The plates were then incubated at 37°C for 24 hours. After the incubation time, the plates were examined for the presence of zones of inhibition as a property of antibacterial activity. The zones of inhibition were measured using a ruler. The study was performed in triplicate and the mean values were presented. The results were reported in (Table-2).

## RESULTS AND DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

The preliminary phytochemical screening for methanolic and aqueous extracts of *Ixora coccinea* L. flowers revealed the presence of alkaloids, glycosides, tannins, flavonoids, steroids and carbohydrates. The methanolic extracts of red, pink and orange flowers showed the presence of alkaloids. The aqueous extracts of all four types of *I. coccinea* flowers showed the presence of glycosides and proteins. Saponins and carbohydrates are present in all the four types of flowers in both the extracts. Flavonoids are present only in white flowers of both extracts. Steroids are present in only aqueous extract of white flowers. The methanolic extracts of all four types of *I. coccinea* flowers showed the presence of tannins.

The antibacterial activity of methanolic and aqueous extracts of red, pink, orange and white *Ixora coccinea* L. flowers were evaluated at two different concentrations (25 µL and 50 µL). In the present study all the methanolic extracts of *Ixora coccinea* L. flowers exhibited considerable antibacterial activity against all tested organisms at low concentrations. The results obtained show that the methanolic extracts of red and pink flowers showed more or less similar antibacterial activity with zone of inhibition value ranging from 17-26 mm. They showed high activity against *B. subtilis* while with *E. coli* & *Klebsiella pneumoniae* it was moderate whereas against *S. aureus* low activity was observed. The orange flower extracts showed moderate

activity with zone of inhibition value ranging from 7-21 mm. The white flower extracts showed low activity with zone of inhibition value ranging from 7-14 mm.

The aqueous extracts compared to the methanolic extracts, displayed less inhibitory effects against all the tested bacteria. The red and pink flowers showed antibacterial activity with zone of inhibition value ranging from 7-21 mm whereas orange and white flowers showed relatively smaller zone of inhibition ranging from 7-12mm.

The results of present investigation clearly indicate that the presence of phytoconstituents varies according to the colour of the flowers. The antibacterial activity of the extracts largely depends on the presence of phytoconstituents and the type of solvent used. Moreover, the effectiveness of the extracts varies with its concentration and the kind of bacteria used in the study. Thus, the study ascertains the value of plants used in herbal medicine could be of considerable interest to the development of new drugs.

**Table-1 Phytochemical screening of Red, Pink, Orange and White flowers of *Ixora coccinea* L.**

TEST	ORANGE		RED		PINK		WHITE	
	WATER	METHANOL	WATER	METHANOL	WATER	METHANOL	WATER	METHANOL
<b>ALKALOIDS</b>								
Dragondroff's test	-	-	-	+	-	+	-	-
Mayer's test	-	+	-	-	-	-	-	-
<b>GLYCOSIDES</b>								
Legal's test	+	-	+	-	+	-	+	-
Saponins test	-	+	+	+	+	-	+	-
<b>CARBOHYDRATES</b>								
Molisch test	+	+	+	+	+	+	+	+
<b>GUMS</b>	-	-	+	-	-	-	-	-
<b>PROTEINS</b>								
Xanthoproteic test	+	-	+	-	+	-	+	-
<b>AMINO ACIDS</b>								
Ninhydrin	-	-	-	-	-	-	-	-
<b>FLAVONOIDS</b>								
Lead acetate test	-	-	-	-	-	-	+	+
<b>STEROIDS</b>								
Salkowski test	-	-	-	-	-	-	-	+
<b>TANNINS</b>								
Ferric chloride test	-	+	-	+	-	+	-	+

**Table-2 The Comparative Antibacterial potencies of Red, Pink, Orange and White flowers of *Ixora coccinea* L.**

Methanolic Extracts	Concentration	Zone of Inhibition in mm			
		<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella Pneumoniae</i>
Red	25µl	20	17	19	19
	50 µl	26	20	23	24
Pink	25µl	19	17	18	18
	50 µl	24	19	20	22
Orange	25µl	13	7	9	18
	50 µl	21	10	12	21
White	25µl	7	9	10	9
	50 µl	9	12	14	11
Aqueous Extracts					
Red	25µl	9	12	12	13
	50 µl	12	16	20	21
Pink	25µl	9	12	11	14
	50 µl	13	18	21	22
Orange	25µl	7	8	7	9
	50 µl	12	11	10	11
White	25µl	7	7	9	8
	50 µl	8	10	12	10
DMSO	25µl	--	--	--	--

## CONCLUSION

The findings of this study confirmed the therapeutic potency of red, orange, pink and white *Ixora coccinea* L. flowers. They displayed variable antibacterial activities. These results offer promising lead for the discovery of potent antibacterial compounds in therapeutic use globally. Further study is required to find out the active component of medicinal value.

## ACKNOWLEDGEMENT

We acknowledge profound gratitude to the management of Vignan Pharmacy College, Guntur for providing infrastructure facilities to conduct this work.

## REFERENCES

- 1) Parekh J, Chanda S, "In vitro antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* Kurz. Flower (Lythraceae)". Brazilian Journal Microbiology, 2007; 38, 204-207.
- 2) Benkeblia N, Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*), Turk J. Biol., 2004; 37, 263-268.
- 3) *The Wealth of India*, dictionary of Indian Raw Materials and Industrial products – raw Materials, National Institute of science communication New Delhi 2002; 3, 351.
- 4) Annapurna J, Amarnath PVS, Amar Kumar D, Ramakrishna SV, Raghavan KV. Antimicrobial activity of *Ixora coccinea* leaves. Fitoterapia 2003; 74, 291–93.
- 5) Yasmeen M, Prabhu B, Agashikar N V. "Evaluation of the antidiarrhoeal activity of the leaves of *Ixora coccinea* Linn. in rats". Journal of clinical and diagnostic research 2010; 4:3298-3303.
- 6) W. D. Ratnasooriya, S. A. Deraniyagala, G. Galhena, S. S. P. Liyanage, S. D. N. K. Bathige, J.R. A. C. Jayakody. Anti-inflammatory activity of the aqueous leaf extract of *Ixora coccinea*. Pharmaceutical Biol. 2005; 43, 147 - 152.
- 7) Moni Rani Saha et al., "In vitro free radical scavenging activity of *Ixora coccinea* L. Bangladesh" J Pharmacol 2008; 3: 90-96.
- 8) Seethadevi B, Nair CRS, Paniker PN. Ancient Sci Life 1986; 107.
- 9) B.S. Nayak et al. "Effect of *Ixora coccinea* flowers on dead space wound healing in rats". Fitoterapia, 1999; 70, 233-236.
- 10) C. Y. Ragasa, F. Tiu, J. A. Rideout. New cycloartenol esters from *Ixora coccinea*. Nat Prod Res, 2004; 18, 319-23.
- 11) Kokate CK, Purohit AP, Gokhale SB, Pharmacognosy, 39<sup>th</sup> Edition, Nirali Prakashan, Pune, 2005:607-611.
- 12) Trease GE, Evans WC. Pharmacognosy, 12th ed. London, Baillieere Tindal, 1983

## AUTHORS AFFILIATIONS AND ADDRESS FOR CORRESPONDENCE

Sowjanya Pulipati\*, Assistant Professor, Vignan Pharmacy College, Vadlamudi, Guntur,A.P