

Research Article

## Antibacterial Activity of the Leaf Extracts of Different Varieties of *Ixora coccinea* Linn: A Comparative Study

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### ABSTRACT

The antibacterial property of leaves of different colored varieties of *Ixora coccinea* Linn (Family: Rubiaceae) was studied against drug-resistant pathogenic bacteria by agar well diffusion and broth dilution methods. The ethyl acetate extracts of all varieties showed significant concentration-dependent antibacterial activity, particularly, against gram negative bacteria. Bioassays showed the presence of several specifically active compounds at different R<sub>f</sub> values in *Ixora coccinea* red plant leaf extract. Presence of phytochemicals such as alkaloids, phenols, steroids, saponins, and flavonoids were observed in the plant extracts which have antibacterial activity and can be used for medicinal purpose. Further mechanistic studies could prove this plant as an excellent source of antibiotic agents.

**Keywords:** *Ixora coccinea*, bioassays, antibacterial activity

### INTRODUCTION

Nature has a source for several medicinal components that favor human for thousands of years and an impressive number of modern drugs have been isolated from such a natural sources; most of these modern drugs are isolated from the traditionally used ones. This plant-based medicine system is playing an essential role in the healthcare about 80% of the world's inhabitants rely mainly on the traditional medicines for their primary health care (Owolabi *et al.*, 2007). Historically, the pharmacological screening of compounds either from natural or synthetic origin are the sources of innumerable therapeutic agents. Random screening is a method for discovering new biologically active molecules and is more productive in the field of antibiotics (Kroschwitz *et al.*, 1992). Medicinal plants represent a rich source of antimicrobial agents. Plants are used as medicines in different countries and are a source of many potent and powerful drugs (Srivastava *et al.*, 1996). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated (Balandrin *et al.*, 1985). Thus considering plants as an excellent source for antimicrobial activity, a systematic examination was done to screen the local flora, *Ixora coccinea* Linn., for its antibacterial activity.

*Ixora coccinea* Linn., (Rubiaceae) is known as the Jungle of Geranium (or) Flame of the woods or vetchi in Ayurvedha. It is a common flowering shrub native to Asia. Its name is derived from an Indian deity. Although there are some 400 species in the genus *Ixora*, only a few are commonly cultivated. *I. coccinea* is commonly called

as *Ixora*, it is a dense, multibranched ever green shrub grows 4–6 feet (1.2-2m) height but sometimes capable of reaching up to 12 feet (3.6m) height. It is traditionally used as hepatoprotective, chemoprotective, antimicrobial, antioxidant, antinociceptive, antimitotic, and antiinflammatory agents. Decoction of roots used for nausea, hiccups, and anorexia; powdered roots used for sores and chronic ulcers. In Indochina, root decoction also used to treat urinary disorders, poultice of fresh leaves and stems of the plants are used to treat sprains, eczema, boils, and contusions. Hence the plant is rich in bioactive constituents and potential therapeutic activities, it has been chosen for the further studies.

### MATERIALS AND METHODS

**Plant Material collection and extraction:** The fresh plants of *Ixora coccinea* Linn (*Ixora coccinea* dwarf, *Ixora coccinea* red, *Ixora coccinea* white, and *Ixora coccinea* yellow) were collected and the same were verified and authenticated botanically by Mr. S. Aroumougame, University of Madras, Chennai. The leaves were then separated from the stem, carefully washed with the tap water, rinsed with the distilled water, and air-dried for 1 hour. The leaves were shade-dried in room temperature for a week. Dried leaves were then ground into powder and subjected to extraction with different solvents such as, hexane, ethyl acetate and methanol (Eloff, 1998a).

**Antibacterial Activity:** The crude extracts were subjected to antibacterial screening against *Pseudomonas aeruginosa* MTCC 2297, *Salmonella typhi* MTCC 733, *Micrococcus luteus* ATCC 4698 and *Vibrio cholerae* ATCC 14035.

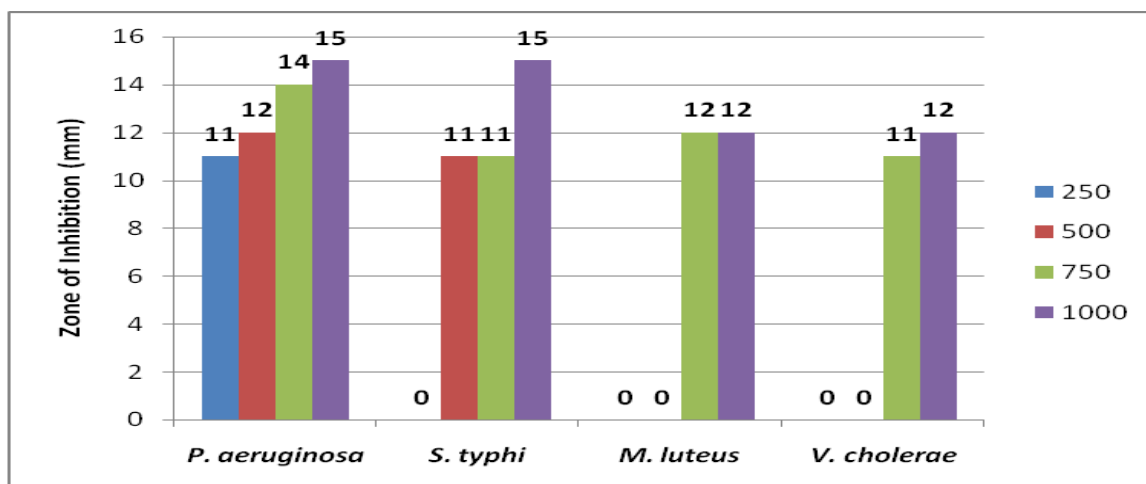


Fig. 1: Antibacterial activity of *I. coccinea* Linn. (Dwarf) Ethyl acetate extract

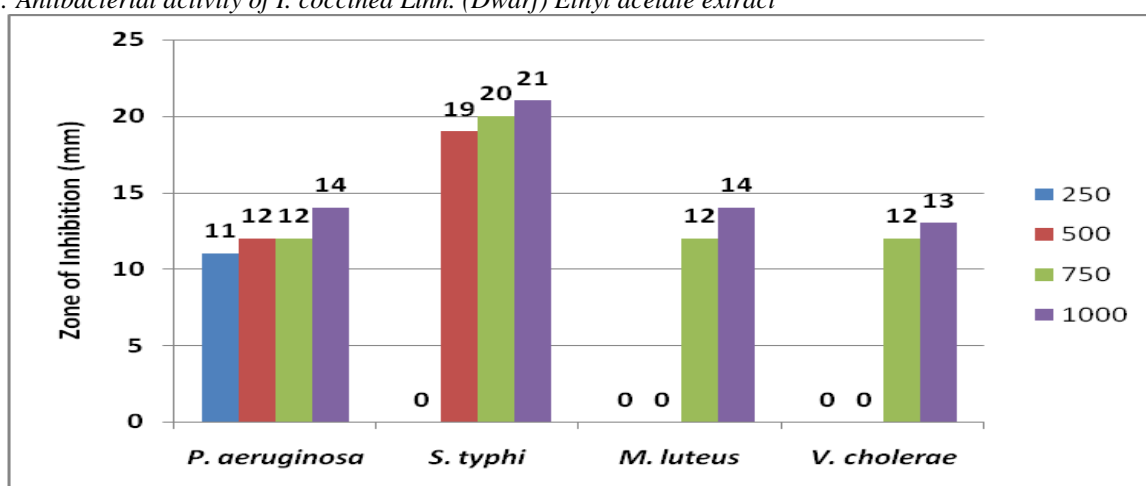


Fig. 2: Antibacterial activity of *I. coccinea* Linn. (White) Ethyl acetate extract

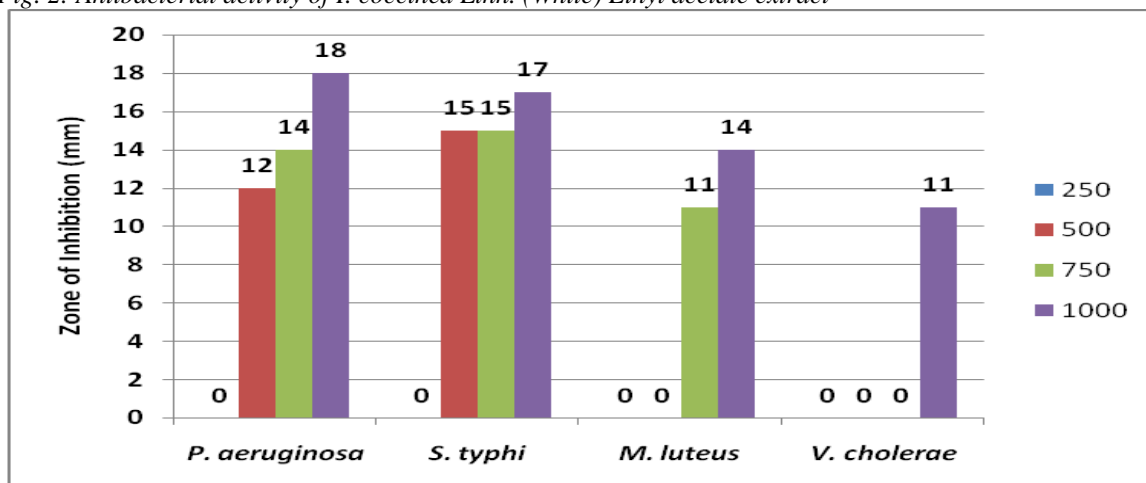


Fig. 3: Antibacterial activity of *I. coccinea* Linn. (Red) Ethyl acetate extract

Well Diffusion Assay: Nutrient agar was prepared and poured in the Petri plate. 24-hours growing cultures (*Pseudomonas aeruginosa*, *Salmonella typhi*, *Micrococcus luteus* and *Vibrio cholerae*) were swabbed on it. The wells (10mm diameter) were made using cork borer and different concentrations of the crude extract were loaded into the wells. The plates were then incubated at 37°C for 24 hours. The inhibition zone diameter was then measured.

Broth Dilution Assay: 5ml of the nutrient broth, 0.1ml of the 24-hours growing cultures (*Pseudomonas aeruginosa*, *Salmonella typhi*, *Micrococcus luteus* and *Vibrio cholerae*) and different concentrations of the drug (100µg–1000µg in Dimethyl sulphoxide) were added into the test tubes and incubated at 37°C for 24 hours. The optical densities were measured spectrometrically at 600nm. The percentage of viable cells was calculated using the formula.

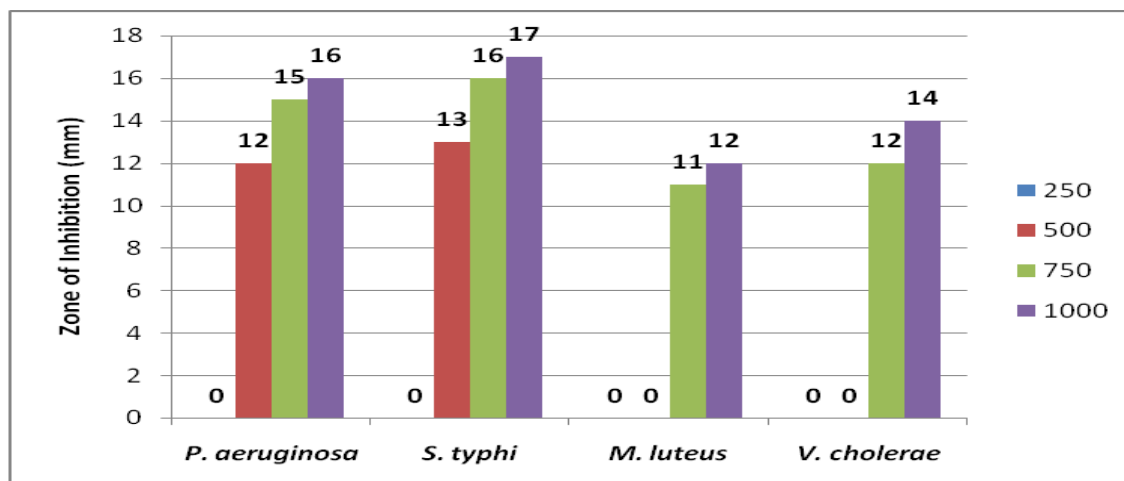


Fig. 4: Antibacterial activity of *I. coccinea* Linn. (Yellow) Ethyl acetate extract

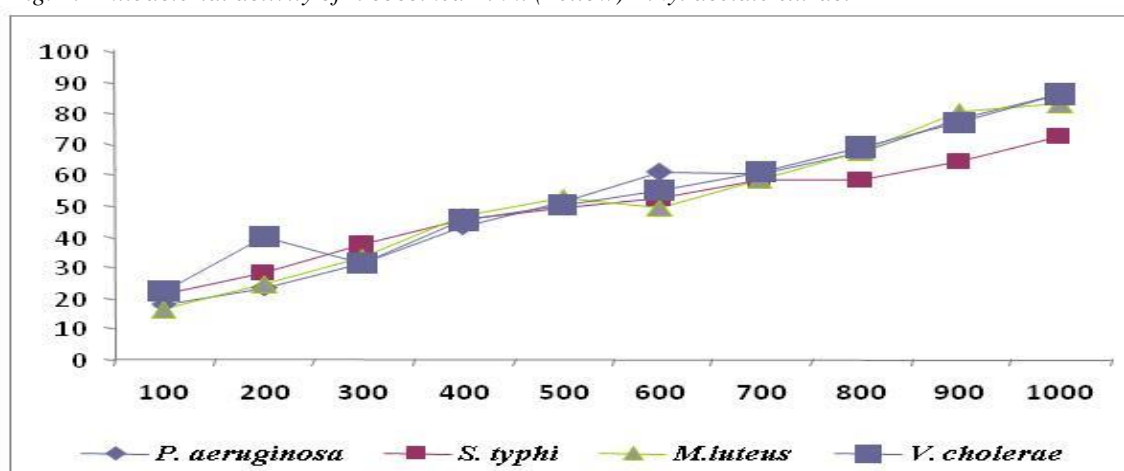


Fig. 5:  $IC_{50}$  values of *Ixora coccinea* Linn. by broth dilution

% viable cells =  $[(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] * 100$

O.D – Optical Density

Thin Layer Chromatography: The ethyl acetate extract of *Ixora coccinea* red was loaded on a precoated silica plate which was then developed using the solvents ethyl acetate and hexane in the ratio 5:5. The spots were identified using UV light, far light and iodine chamber. Then  $R_f$  value was calculated by the ratio of distance traveled by the solute to the distance traveled by the solvent.

Bioautography: Bioautography is a rapid aid in the bioassay-guided isolation and fractionation of antibacterial compounds and fractions (Yff *et al.*, 2002). In this method, the activity of plant extract against bacteria is determined on chromatograms, in accordance with the bioautography procedure of Begue and Kline (1972). Developed chromatography plates of crude extract were dried overnight, sprayed with a suspension of actively growing *Salmonella typhi* incubated at 37°C in a chamber at 100% relative humidity for 18 hours. Plates were sprayed with MTT (5 mg/ml). Clear zones on the chromatogram indicate inhibition of the growth after incubating for 4 h at 37°C. This method was chosen for its simplicity, low cost, accuracy, and rapid results, make it ideal for bioassay-guided isolation.

Phytochemical Screening of *Ixora coccinea* (Red) Crude Extract: Qualitative phytochemical tests for the identification of alkaloids, phenols, flavonoids, terpenoids, steroids, and saponins were carried out for all the extracts by the method described by Harborne, JB (1998). The freshly prepared extracts of *Ixora coccinea* red were qualitatively tested for the presence of chemical constituents.

## RESULTS AND DISCUSSION

**Antibacterial Activity:** *In vitro* preliminary screening was done to evaluate the antibacterial activity of different coloured varieties of the plant leaf extracts of *Ixora coccinea* Linn against drug-resistant pathogenic bacterial cultures, using different solvents by agar well diffusion method. Among various solvents used, the ethyl acetate extracts of four varieties showed high-level inhibition. The zone of inhibitions of the four different coloured plant leaves are shown in Figs. 1–4.

Among four plant leaves, the *Ixora coccinea* red variety shows maximum inhibition against the pathogens and chosen to identify its inhibitory concentration 50 ( $IC_{50}$ ) by broth dilution method. The  $IC_{50}$  concentration of ethyl acetate extract of *I. coccinea* red was ranged from 500 to 600  $\mu\text{g/ml}$ , which was dose-dependent with respect to

different test pathogens (Fig. 5). In the work done by Kameda *et al.*, (1987), dichloromethane and aqueous extracts from the leaves as well as ethyl acetate extract from the flowers showed antibacterial activity against *Staphylococcus aureus*.

The chromatogram developed with ethyl acetate and hexane in the ratio 5:5(v/v) revealed the presence of six major compounds at the R<sub>f</sub> value of 0.61, 0.69, 0.79, 0.82, 0.87, and 0.95, respectively, viewed under iodine vapor and UV illumination. *I. coccinea* red showed antibacterial

Table 1: Preliminary phytochemical screening of the leaf extract of *I. coccinea* red

S.No	Constitutions	Methanol extract
1	Alkaloids	+++
2	Phenols	++
3	Flavonoids	++
4	Terpenoids	-
5	Steroids	+++
6	Saponins	++

activity against *Salmonella typhi* by direct bioautography. The zone of inhibition was estimated at R<sub>f</sub> value 0.95.

The phytochemical analysis on *I. coccinea* red extract revealed the presence of alkaloids, phenols, flavonoids, steroids, and saponins and is shown in Table 1. In the previous study, screening of ether extract of *I. coccinea* was done which confirmed the presence of alkaloids, flavonoids, saponins, steroids, terpenes and from the methanol extract, alkaloids, phenols, and steroids were confirmed (Annapurna and Raghavan, 2003).

## CONCLUSION

Extracts of *I. coccinea* red in this study confirmed a broad spectrum of antibacterial activity against both Gram positive and Gram negative bacteria. This activity is maybe due to the presence of alkaloids, phenols, flavonoids, steroids and saponins identified which further confirm its application in the folklore medicine. Bioactive substances from this plant can, therefore, be employed in the formulation of antimicrobial products for the treatment of various bacterial infections. Isolation, identification, and purification of phytoconstituents and determination of their respective antibacterial properties and toxicological evaluation in the view of formulating novel chemotherapeutic agents are the future works to be carried out.

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