

Exploring The Therapeutic Potential of *Ixora coccinea* Linn: Phytochemical, Antioxidant, Anti-Inflammatory, And Antibacterial Studies

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ABSTRACT

Inflammation is a normal process in living organisms that responds to tissue damage and infection, but chronic inflammation can lead to chronic diseases like arthritis, cardiovascular diseases and cancer. As the problem of antimicrobial resistance infections becomes more prevalent, novel plant-based therapeutic agents are required. In the present study we have investigated the pharmacognostic, phytochemical, antioxidant, anti-inflammatory and antibacterial properties of the ethanolic extract of *Ixora coccinea* Linn. All phytochemicals were found in the screening of phytochemicals present in the plant. The total phenolics and flavonoids content were 52.11 mg/g and 78.23 mg/g respectively. Hydrogen peroxide and nitric oxide scavenging assays proved the antioxidant activity with IC₅₀ 86.8 µg/mL and 2.67 µg/mL, respectively, showing good activity against free radicals. The anti-inflammatory activity of the extract was significant *in vitro* and it inhibited the protein denaturation and stabilised human red blood cell membranes in a concentration dependent manner. An antibacterial test showed significant inhibition of *E. coli* and *S. aureus*, with better results against Gram-positive bacteria. Based on the results obtained from the study, it is concluded that *Ixora coccinea* Linn has been traditionally used for medicinal purposes thus giving scientific evidence for the same. It is a natural source that has antioxidant, anti-inflammatory and antibacterial properties. More *in vivo* studies, toxicity tests and clinical trials are needed for safety, effectiveness, and therapeutic use

.Keywords: *Ixora coccinea*, Anti-inflammatory activity, Inflammation, *E. coli*, *S. aureus*, Gram-positive bacteria

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INTRODUCTION

Inflammation is a highly organised response to harmful agents, such as tissue injury, toxic chemicals, and pathogenic microbes. Innate immunity relies on this mechanism to remove infectious agents and start the

healing process. [1] Typically, acute inflammation consists of vascular changes, leukocyte infiltration, and the release of inflammatory mediators that restore tissue homeostasis. Chronic inflammation occurs when the inflammatory response is persistent or out of control,

and it contributes to many diseases such as cardiovascular diseases, diabetes mellitus, neurodegenerative diseases, cancer, and rheumatoid arthritis. [2] Chronic inflammation is closely associated with oxidative stress, which is characterized by increased levels of ROS, pro-inflammatory cytokines, and inflammatory enzymes. [3] Inflammatory diseases are often caused by oxidative stress. The excessive production of hydrogen peroxide, nitric oxide, and superoxide anions overrides the endogenous antioxidant defense system, causing lipid peroxidation, protein oxidation, DNA damage, and inflammation. [4] As a result, antioxidants that neutralize ROS and RNS have garnered a great deal of attention as therapeutic agents in the prevention and management of inflammation-related diseases. Medicinal plants-derived natural antioxidants have gained high popularity due to their wide range of pharmacological properties, reduced toxicity, and better safety profile than many synthetic antioxidants. [5]

However, bacterial infection remains a significant public health problem despite great advances in antibiotic therapy. The pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* cause a wide range of infections in the gastrointestinal tract, urinary tract, skin, respiratory system and bloodstream. [6] A growing number of multidrug-resistant bacteria strains are making drugs less effective, resulting in longer hospital stays, higher health care costs, and more deaths. A lack of antimicrobial agents with different mechanisms of action has led to antibiotic resistance becoming one of the world's greatest public health threats. [7]

Medicinal plants contain phytochemicals with medicinal properties that can be used as effective alternatives or complementary treatments against bacteria resistant to antibiotics. For centuries, medicinal plants have been used in traditional medicine systems such as Ayurveda, Siddha, and TCM, as well as in indigenous medicine systems. [8] The secondary metabolites they contain include flavonoids, alkaloids, tannins, glycosides, saponins, terpenoids, and phenols. There are many pharmacological effects associated with these phytoconstituents, including antioxidant, anti-inflammatory, antimicrobial, anticancer, hepatoprotective and immunomodulatory properties.

Several traditional medicinal claims have been validated by research on phytochemicals and pharmacology, leading to several plant-derived therapeutic agents being developed. Thus, systematic research on medicinal plants is regarded as a critical component of finding natural products that are more effective and safer.

Ixora coccinea Linn Jungle Geranium, can be found throughout the tropics and subtropics (family: Rubiaceae). A variety of plant parts have been used in traditional medicine to treat wounds, skin infections, ulcers, diarrhea, dysentery, fever, inflammatory disorders, and microbial infections. It is well known that flavonoids, phenolic compounds, alkaloids, tannins, glycosides and saponins in *I. coccinea* are antioxidants, anti-inflammatory and antimicrobial components. [9]

It has been demonstrated that the plant has biological properties in some preliminary investigations, but further scientific validation is limited and requires further pharmacognostic, phytochemical, antioxidant, anti-inflammatory, and antibacterial studies. Accordingly, the present study examined the ethanolic extract of plant *Ixora coccinea* Linn for pharmacognostic and phytochemical properties, antioxidant activity, anti-inflammatory properties, and antibacterial properties. [10]

A pharmacognostic evaluation was used to identify the plant material and assess its quality, whereas phytochemical screening and phenolic content estimation were used to assess the presence of the major bioactive constituents. [11] Protein denaturation inhibition and human red blood cell membrane stabilization assays were used to determine antioxidant potential and anti-inflammatory activity. Additionally, *Escherichia coli* and *Staphylococcus aureus* were tested for antibacterial activity of the extract. [12] In this study, the scientific basis for *Ixora coccinea* Linn will be established. In the future, they could be used as sources of antioxidant, anti-inflammatory, and antibacterial agents.

Methodology

Collection of plant materials

Leaves of *Ixora coccinea* Linn. (Rubiaceae) were collected from local areas of Chennai, Tamilnadu. The materials were confirmed (Voucher Specimen-223/2025) by Dr. P.Radhakar, Research Officer (Botany), Central Council for Research in Siddha, Chennai. The flowers were sliced after collection and dried in the well ventilated room under shade for 7 days at room temperature. They were then pulverized in a grinding mill to make a fine powder. The resulting powder was screened (sieve No. 50) to make it uniform and was kept in air tight container for further use.

Extraction process and phytochemical screening

Ixora coccinea linn. flowers were coarsely powdered, extracted with ethanol (60–80°C) in a Soxhlet extractor, and concentrated using rotary vacuum evaporators. A preliminary analysis of the extract's was performed

prior to more detailed phytochemical analysis as per standard procedure. [13]

Determination of Total Phenolic content

The total phenolics present in the extract of *Ixora coccinea* Linn. obtained in ethanolic extract. The Folin–Ciocalteu method was used to determine (EEICL). The solutions of gallic acid and EEICL were prepared in 10 mL volumetric flasks of standard concentration (10–50 µg/mL). Distilled water (5 mL) and Folin–Ciocalteu (0.5 mL) reagent were added to each flask and thoroughly mixed. This after 5 min 1.5 mL of sodium carbonate solution was added and the volume was increased to 10 mL by adding distilled water. The reaction mixtures were allowed to stand at room temperature for 2 h for colour development. Absorbance readings were taken at 760 nm using a UV–Visible spectrophotometer with a reagent blank. Total phenolic content was determined using a gallic acid calibration curve (mg/g gallic acid equivalents (GAE)). [14]

Determination of Total Flavonoid content

The ethanolic extract of *Ixora coccinea* Linn. has a total of flavonoids. The aluminum chloride colorimetric method was used for determining (EEICL). Ten millilitres of volumetric flasks were used to prepare quercetin and EEICL solutions (10–50 µg/mL) in standard manner. 4 mL of distilled water and 0.3 mL of 5% sodium nitrite were placed in each flask. The sample was treated with 0.3 mL of the 10% aluminum chloride solution after 5 min after which 2 mL of the 1 M sodium hydroxide solution was added after 1 min. The volume was made up to 10 mL with distilled water and shaken thoroughly. Absorbance was determined at 510 nm wavelength in UV–Visible spectrophotometer by taking a reagent blank. The total flavonoids were determined using a calibration curve of quercetin and reported as mg of quercetin equivalents per gram of extract (mg QE/g). [15]

In vitro Antioxidant Activity

Hydrogen peroxide scavenging assay

The hydrogen peroxide scavenging activity of the ethanolic extract of *Ixora coccinea* Linn. (EEICL) was evaluated by mixing 1 mL of the test solution with 3.8 mL of 0.1 M phosphate buffer (pH 7.4) and 0.2 mL of hydrogen peroxide solution. The reaction mixture was incubated for 10 min, and the absorbance was measured at 230 nm using a UV–Visible spectrophotometer. A reaction mixture without the sample served as the blank, while a sample blank without reagents was also prepared. Ascorbic acid was used as the standard antioxidant. [16]

Nitric oxide Scavenging assay

The nitric oxide radical scavenging activity of the ethanolic extract of *Ixora coccinea* Linn. (EEICL) was evaluated using sodium nitroprusside. Different concentrations (2–10 µg/mL) of EEICL and ascorbic acid (standard) were prepared in phosphate-buffered saline (PBS) to a final volume of 1 mL, followed by the addition of 2 mL of 10 mM sodium nitroprusside. The reaction mixtures were incubated for 150 min at room temperature. Subsequently, 0.5 mL of the incubated solution was mixed with 1 mL of 0.33% sulphanilamide and allowed to stand for 5 min, followed by the addition of 1 mL of 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride (NED). After incubation for 30 min at room temperature, the absorbance was measured at 516 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as the standard, and the percentage nitric oxide radical scavenging activity was calculated. [17]

In vitro Inflammatory Activity

Different concentrations (6.25–500 µg/mL) of the ethanolic extract of *Ixora coccinea* Linn. (EEICL) and diclofenac sodium (standard) were prepared. An aliquot (0.5 mL) of each solution was mixed with 2.5 mL phosphate-buffered saline (PBS) and 2 mL egg albumin. The mixtures were incubated at 27°C for 15 min, followed by heating at 70°C for 10 min. After cooling, absorbance was measured at 660 nm using a UV–Visible spectrophotometer against distilled water as the blank. The reaction mixture without the test sample served as the control, and the percentage inhibition of protein denaturation was calculated. [18]

Human Red Blood Cell (HRBC) Membrane Stabilization Method

In the HRBC Membrane Stabilization Method, the extract inhibited the lysis of membranes caused by hypotonicity at concentrations of 100µg/ml and 200µg/ml, as compared to diclofenac sodium at concentrations of 10µg/ml and 25µg/ml respectively. [19]

In vitro Anti-Bacterial Activity

Disc diffusion study on *Escherichia coli*

The ethanolic extract of *Ixora coccinea* Linn. showed an antibacterial activity. The disc diffusion method was used to test (EEICL). The test bacterial strains were inoculated onto nutrient agar plates containing different concentrations of EEICL (5, 10, 15 and 20 µg/mL) in the form of sterile discs. Vancomycin (30 µg/disc) was used as the standard and a saline loaded disc was used as control. The plates were incubated for 24 h at 37° and the antibacterial activity was estimated by measuring the diameter of the zone of inhibition (mm). [20]

Disc diffusion study on *Staphylococcus aureus*

The test bacterial strains were spread on agar plates to which the (30 µg/disc) were added. A standard of 30 µg/disc vancomycin was used and a saline loaded disc was used as the control. The plates were then incubated at 37°C for 24 hours and the antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm). [21]

Statistics

One-way ANOVA was run on GraphPad Prism (version 5), the post-test Tukey's multiple comparison was used. The results are presented as percentage. The data are presented as mean ± S.E.M and were considered to be significant at $P < 0.001$.

Results

Secondary metabolites screening of plant extracts

Preliminary phytochemical screening of the ethanolic extract of *Ixora coccinea* Linn. revealed the presence of several important bioactive constituents, including alkaloids, glycosides, flavonoids, tannins and phenolic compounds, saponins, carbohydrates, and proteins/amino acids. However, steroids and triterpenoids, gum and mucilage, and fixed oils and fats were absent. These findings indicate that the extract is rich in phytochemicals that may contribute to its antioxidant, anti-inflammatory, and antibacterial activities.

Total Phenolic content determination

Total phenols in the Ethanolic Extract of *Ixora coccinea* Linn. The method used for determining (EEICL) was the Folin–Ciocalteu method with gallic acid as a reference standard. The absorbance of gallic acid and EEICL was found to be concentration dependent (10–50 µg/mL) showing the existence of phenolic compounds in the extract. The total phenolic content of EEICL was determined to be 52.11 mg gallic acid equivalents/g of extract using the calibration curve of gallic acid, indicating the great amount of phenolics in EEICL extract which may have antioxidant and anti-inflammatory potential.

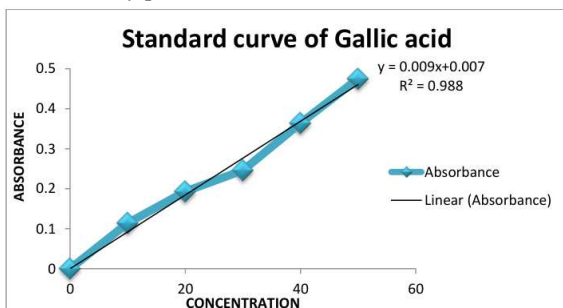


Figure: 1 Standard Calibration curve of gallic acid

Total Flavonoid content determination

Quercetin was used as the reference standard and the (EEICL) was obtained by the colorimetric method with aluminium chloride. The absorbance of both quercetin and EEICL was found to increase with the increase in concentration level of the extracts (10–50 µg/mL), suggesting the presence of flavonoids in the extract. The total flavonoids content of EEICL was determined as 78.23 mg of quercetin equivalents (QE)/g extract, based on calibration curve of quercetin and it was found that the extract is rich in flavonoid compounds which may be responsible for potent antioxidant, anti-inflammatory and antibacterial activity.

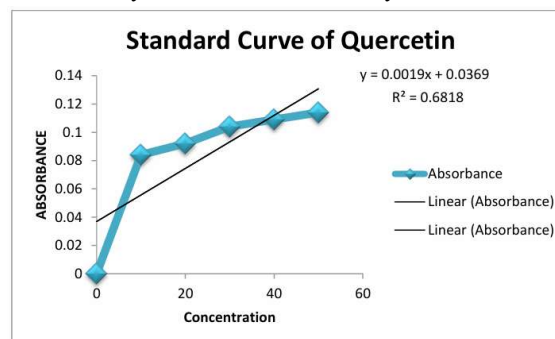


Figure: 2 Standard Calibration curve of Quercetin

In vitro Antioxidant activity

Hydrogen Peroxide Scavenging Activity

Ethanolic extract of *Ixora coccinea* Linn. was found to have hydrogen peroxide scavenging activity. (EEICL) was found to be concentration dependent (20–100 µg/mL). Standard ascorbic acid showed higher percentage inhibition at all the tested concentrations while EEICL showed good antioxidant activity with an IC₅₀ value of 86.8 µg/mL which is slightly less than that of ascorbic acid (90.99 µg/mL) suggesting good hydrogen peroxide scavenging potential.

Nitric Oxide Scavenging Activity (NOSA)

Scavenging activity of nitric oxide radical of EEICL also enhanced as per the increase in concentration (2–10 µg/mL). EEICL had a

significant nitric oxide scavenging activity with IC_{50} value of $62.31\mu\text{g/mL}$ while ascorbic acid had IC_{50} value of $86.16\mu\text{g/mL}$. The results suggest that EEICL has potent nitric oxide radical scavenging activity which makes it a natural antioxidant.

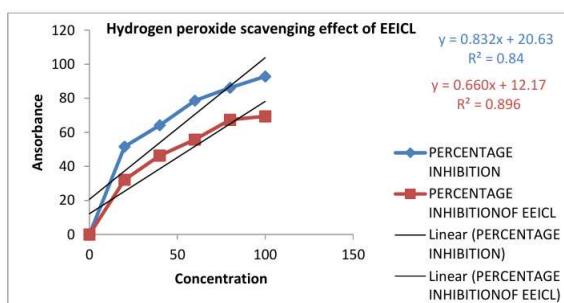


Figure: 3 Hydrogen peroxide scavenging effect of EEICL

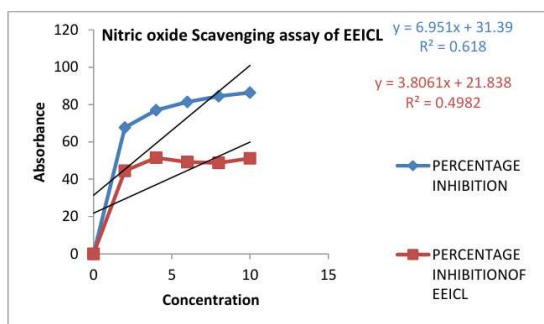


Figure: 4 Nitric oxide Scavenging assay of EEICL

In vitro inflammatory Activity (Protein denaturation)

The anti-inflammatory activity exhibited by (EEICL) was concentration dependent in membrane stabilization assay. The percentage inhibition gradually increased from 5.46% at $6.25\mu\text{g/mL}$ to 33.91% at $50\mu\text{g/mL}$ suggesting the increased the membrane stabilizing activity with the increase in concentration. EEICL was able to show a significant anti-

inflammatory activity, even though the standard drug was more effective in all the concentrations, it is a clear indication of the ability of EEICL to protect the erythrocyte membranes from hypotonicity induced lysis.

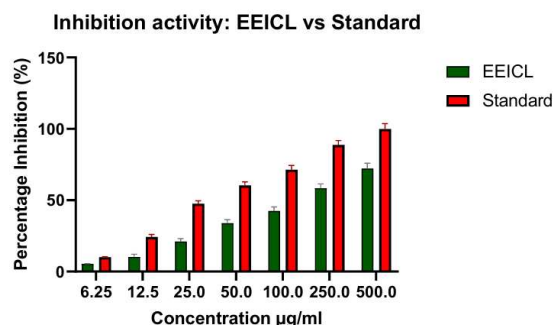


Figure 5; Standard Inhibition vs EEICL Inhibition

Human Red Blood Cell Membrane Stabilization Method

The ethanolic extract of *Ixora coccinea* Linn. showed that the extract of the plant can stabilize the erythrocyte membrane. EEICL showed concentration dependent anti-inflammatory activity. The membrane stabilization effect was more prominent in higher concentrations of extract, reflecting the higher protection from the lysis of the erythrocyte membranes. While the standard drug exhibited higher activity at all the concentrations tested, EEICL exhibited significant membrane stabilizing property, indicating that anti inflammatory activity could be due to flavonoids and phenolics found in EEICL. The results of this work are in agreement with the traditional application

of *Ixora coccinea* Linn. in the treatment of inflammatory diseases.

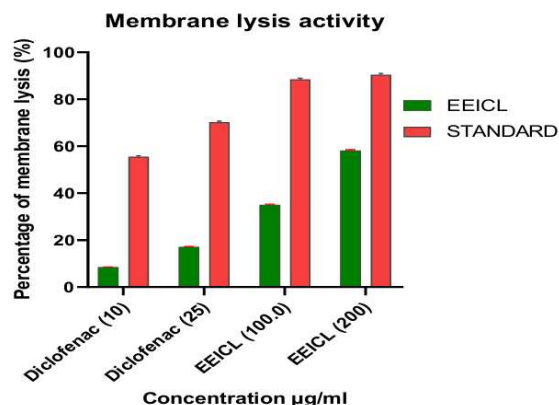


Figure: 6 Membrane lysis activity of Standard vs EEICL

In vitro anti-bacterial activities

Disc diffusion method on *Escherichia coli* and *Staphylococcus aureus*

The ethanolic extract of *Ixora coccinea* Linn. Concentration dependent antibacterial activity against *Escherichia coli* was observed for (EEICL) with the zone of inhibition increasing from $4.23 \text{ mm} \pm 0.33$ to $11.09 \text{ mm} \pm 1.66$ with increasing concentration from 5 µg/mL to 20 µg/mL of antibiotic. The activity of EEICL was lower than that of standard drug ($16.34 \pm 2.29 \text{ mm}$) but it showed decent antibacterial activity. EEICL exhibited very strong antibacterial activity against *Staphylococcus aureus* with $21.65 \pm 2.19 \text{ mm}$ zone of inhibition which was comparable to standard ($22.42 \pm 2.38 \text{ mm}$). The results indicated that EEICL has good antibacterial activity in particular, against Gram +ve bacteria, which

indicated its potential as a natural antimicrobial agent.



Figure: 7 Zone of inhibition of *Escherichia coli* (Gram negative) by Disc diffusion method



Figure: Zone of inhibition of *Staphylococcus aureus* (Gram Positive bacteria) by Disc diffusion method

Discussion

Chemical screening of EEICL showed the presence of alkaloids, flavonoids, glycosides, tannins, phenols, and saponins. The results obtained in the present study are in line with the studies carried out by who reported the same phytoconstituents present in *Ixora coccinea*. [12] These bioactive compounds may underlie antioxidant, anti-inflammatory, and antibacterial properties observed. The

total phenolic content of EEICL was 52.11 mg GAE/g and the total flavonoid content was 78.23 mg QE/g, which shows that EEICL is a good source of polyphenols. The study was reported, where they showed that extracts of *I. coccinea* have high phenolic and flavonoid contents and these are responsible for its antioxidant properties. [19] EEICL exhibited strong hydrogen peroxide and nitric oxide scavenging activities, providing evidence for its antioxidant properties. The findings are similar to those reported, where the antioxidant activity of *I. coccinea* was found to be due to its high content of phenolic and flavonoid compounds that can scavenge the free radicals of the Reactive Oxygen Species (ROS). [14] EEICL showed protein denaturation inhibitory activity, which was concentration dependent, showing a significant anti-inflammatory activity. This was reported in similar works, and the flavonoids and phenolic composition were responsible for the inhibitory effects of *I. coccinea* extracts on protein denaturation. [20] The dose dependent stabilization of HRBC membrane was observed in the extract which indicated that it can prevent membrane lysis during inflammation. The results are comparable to the results of flavonoid rich crude extracts of medicinal plants reported with their membrane stabilizing property via protection of lysosomal membrane. [16] EEICL exhibited concentration-dependent

antibacterial activity against *Escherichia coli* and exhibited strong antibacterial activity against *Staphylococcus aureus*, which showed more effect against Gram-positive strain. A similar finding was reported by who found that extracts of *I. coccinea* showed greater effect on Gram-positive bacteria than on Gram-negative bacteria because of difference in permeability of cell wall. [21]

Conclusion

The results of present study reveal that the ethanolic extract of *Ixora coccinea* Linn. has remarkable antioxidant, anti-inflammatory and antibacterial potential with the abundance of phytochemicals such as phenolics and flavonoids. The extract exhibited good free radical scavenging activity, protein denaturation inhibition, membrane stabilization, and significant antibacterial activity particularly against *S. aureus*. The findings are scientific proof for the traditional medicinal use of *Ixora coccinea* Linn. and could also be a source of bioactive compounds that could be used in developing therapeutic agents. Still, further phytochemical characterization, *in vivo* pharmacological studies, toxicological assessment and clinical studies are necessary for confirmation of its safety and therapeutic efficacy.

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