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Phytochemical Profiling, Chromatographic Analysis and Biological Evaluation of Flower Extract *Ixora Coccinea*

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Abstract: In the Indian traditional Ayurvedic medical system, plants from the genus Ixora are used to treat a wide range of illnesses. The study extracted the Ixora coccinea plant, analyzed the plant extracts both qualitatively and quantitatively, and measured the antioxidant activity using the DPPH method. Initial qualitative phytochemical screening provides an indication of the herb's potential for medicinal use. Ixora coccinea shows promise as a potential therapeutic lead as drug resistance becomes a greater concern in contemporary pharmacotherapeutics. Research on this flower is essential since its bioactive components may offer substitute treatment alternatives for treating drug-resistant infections and a range of human illnesses. Ixora coccinea phytochemical analysis is crucial to examine its therapeutic qualities and confirm that it works as a natural cure. The results of the study's screening for bioactive components that give plants their biologically active characteristics guaranteed the presence of the following phytochemical parameters: amino acids, phenols, alkaloids, flavonoids, terpenoids, coumarins, tannin, saponin, anthocyanin, and anthraquinone.

Keywords: *Ixora Coccinea, Phytochemical Screening, DPPH assay, Chromatographic techniques, Antioxidant.*

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I. INTRODUCTION

A member of the Rubiacea family, *Ixora coccinea* Linn. Is an evergreen shrub that may be found all throughout India. It is also referred to as the jungle of geranium and red *Ixora.* The flowers, leaves, roots, and stems are used in many traditional medicines in the Indian Traditional Medical System, or Ayurveda, to treat a variety of illnesses. One the popular decorative plant *Ixora coccinea,* also known as *Ixora coccinea* (IC), is a member of the Rubiaceous family and is distinguished by its eye-catching red blossoms. *Ixora coccinea* flower extract has not yet been thoroughly investigated for its potential medical use, particularly in the treatment of infections, skin disorders, and oral health problems. This study's main goal is to assess the pharmacological characteristics of *Ixora coccinea* flower extract, such as its antioxidant and antibacterial qualities. The goal of the study is to separate and pinpoint the active ingredients causing these effects. Additionally, this extract's possible application in formulations for skin and oral care will be investigated (1).

Ixora coccinea is a perennial evergreen shrub with a dense, rounded growth habit and a taproot system. It usually grows to a height of three to six feet and spreads to a comparable width. They vary in terms of flower size and color, plant height, and leaf size. The leaves are rectangular and obtuse, opposite, glossy, dark green, and sessile or sub-sessile. They are usually seen in pairs along the stem and range in length from 3 to 6 inches. (2)

Table 1 *Ixora coccinea* is a Perennial Evergreen

Kingdom	**Plantae**
Division	Tracheophyta
Class	Magnoliopsida
Order	Gentianales

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Family	Rubiaceae
Genus	*Ixora*
Species	*coccinea*

➢ *Plant Taxonomy*

Fig 1 Ixora Coccinea Plant

The little shrub *Ixora coccinea* Linn (Rubiaceae), which is grown throughout India, has been said to have a number of therapeutic uses. The evergreen shrub *Ixora coccinea* Linn is dense, multibranched, and can reach a height of 12 feet (3.6 meters), however it usually stands 4-6 feet (1-2-2 meters) tall. "pokokTudungPeriuk" is the Malay term for *Ixora coccinea* Linn. Traditional medical use of *Ixora coccinea* Linn has been demonstrated for a number of ailments, including chemo protective, antibacterial, antioxidant, antinociceptive, hepatoprotective and anti-inflammatory qualities. (3) With the exception of a recent study that demonstrated the floral extract's strong antioxidant qualities, there are little studies on *I. coccinea's* antioxidant activity. This was explained by the flower's high concentration of hydrophilic phenolic chemicals. Given this, we provide the antioxidant activity (AOA) and total phenolic content (TPC) of *I. coccinea's* flower, leaf, and stem. (4)

A genus of flowering plants belonging to the Rubiaceae family is called *Ixora*. With its centre of variety in Tropical Asia, it is home to about 500 species and is made up of tropical evergreen trees and shrubs. In the US, *Ixora* is also frequently seen in subtropical regions like Florida (5).

According to Pharmacological reports, the leaves have antibacterial properties. By extending the lifespan of treated mice, the flowers exhibit chemo protective benefits against cyclophosphamide-induced damage. It has antioxidant properties as well. In rats, dead space wounds can be healed by flowers. According to reports, the blooms contain anti-inflammatory properties similar to those of indomethacin. There have been reports of the plant's leaves having anti-diarrheal properties. Mice have demonstrated antinociceptive action in response to *I. coccinea* L. aqueous leaf extract. (6)

Small to medium-sized, hardy shrubs like *Ixora coccinea* (Family: Rubiaceae) are grown for their aesthetic qualities and are also used in traditional Indian medicine. IC has been documented to have a variety of pharmacological characteristics, such as antimitotic and anti-inflammatory effects. It has been discovered that *IC* leaves and flower extracts have antibacterial properties. Ursolic acid and triterpenoid are present in flower extract. Two novel cycloartenol esters, lupeol fatty ester, lupeol, oleanolic acid, and sitosterol were produced by the flowers. (7)

Medicinal plants have been utilized as antimicrobial agents since ancient times. Many nations throughout the world employ the chemical components found in the many species of *Ixora* in the Rubiaceae family as ethnobotanical plants. Anticancer, anti-inflammatory, anti-diarrheal, anti-asthmatic, anti-ulcer, hypotensive, antiviral, and antinociceptive properties have all been documented for the leaves of this plant. The leaves, flowers, root, and frits have historically been used to treat a variety of illnesses. (8)

II. MATERIALS AND METHODS

Chemicals Used: Ethanol, n-hexane, Potassium dihydrogen orthophosphate, Potassium ferricyanide, Trichloro acetic acid, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Disodium hydrogen orthophosphate, Sodium hydroxide, concentrated Hydrochloric acid, Ferric chloride (FeCl3), Benzene, Hexane, Methanol, Chloroform, Ethyl acetate, Petroleum ether ,acetonitrile, 0.1 % phosphoric acid (1,1 -Diphenyl-2 picrylhydrazy, ascorbic acid, Nutrient Agar , Dimethyl, Gallic acid, phenols reagent ,tannins reagent , Lead acetate, Sulphuric acid, Benedict's, Di-Methyl Sulph-Oxide, Mueller-Hinton agar etc.

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➢ *Collection of Plant Material.*

Fresh leaves & flowers from the University of Agricultural Science, Dharwad, Karnataka were gathered, cleaned, and allowed to dry in the shade for roughly 10 days. The dehydrated foliage was ground into a coarse powder. (8)

➢ *Preparation of Plant Extract.*

Fresh flowers were professionally cleaned with water to get rid of any dirt and debris. And put through solvent extraction processes with methanol, petroleum ether, chloroform, and other chemicals. The extracts were kept for later examination in a refrigerator. (3)

Fig 2 *Ixoracoccinea* leaf & flower powder.

(A) (B)

Fig 3 Extraction by soxhlet apparatus (A) Extraction of flower (B) Extraction of leaf

➢ *Qualitative Phytochemical Analysis:*

- *Detection of Alkaloids:*

✓ **Test for Dragendorff's reagent:** A few ml of filtrate were mixed with one or two ml of Dragendorff's reagent. A noticeable red precipitate showed that the test was positive.

✓ **Test for Mayer's:** A drop or two of the Mayer's reagents were added to a few ml of filtrate via the test tube's sidewalls. A creamy white precipitate showed that the test was successful.

- *Detection of Flavonoids:*

✓ **Test for Alkali reagent:** The presence of flavonoids was confirmed by treating an aqueous solution of the extract with a 10% ammonium or sodium hydroxide solution, which resulted in the development of an intense yellow coloration.

✓ **Test for Lead acetate:** When lead acetate solution is added to the extract, flavonoids are present because a yellow precipitate forms.

- *Detection of Glycosides:*

✓ **Test for Keller-Killiani:** An aqueous solution of the extract was treated with strong acid & ferric chloride, formation of reddish brown colour, the presence of digitoxose and, therefore, cardiac glycosides.

- *Detection of Tannin:*

✓ **Test for Ferric chloride:** 50 ml of the extract was dissolved in 5 ml of distilled water. This was mixed with a ferric chloride solution, blue-green or deep blue-black

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indicates the presence of tannins and phenolic compounds.

- *Detection of Saponins:*

✓ **Test for Frothing test:** To test for the presence of Saponins, 50 mg of the extract was diluted with distilled water to a final volume of 20 mL. The resulting suspension was shaken vigorously in a graduated cylinder for 10 minutes. The formation of a stable two-centimeter layer of foam indicated the presence of saponins.

- *Detection of Terpenoids:*

✓ **Test for Salkowski:** In this test, concentrated sulfuric acid is combined with an extract, typically containing chloroform. A good result for terpenoids is shown by a reddish-brown colour at the interface between the two layers.

- *Detection of Polyphenol:*

✓ **Test for Ferrozine:** An aqueous solution of the extract was treated with, in order to maintain pH; a buffer (such as ammonium acetate) and a known quantity of ferrozine solution are added to the sample, formation of raddish blue (12).

➢ *Chromatographic Analysis:*

- *Thin Layer Chromatography:*

A solvent system consisting of petroleum ether, chloroform, and methanol (1:0.5:0.1, V/V/V) was utilized to develop the exudates on silica gel plates, specifically silica gel 60 F_{254} (10x20 cm, 0.2mmlayer), after the polyphenol-rich fraction from *Ixoracoccinea* flowers was loaded onto pre-coated TLC ($60F_254$). It has a visible and non-visible spot and is fluorescent with UV light at 240 and 360 nm. (9)

- *High Performance Liquid Chromatography:*

One of the most significant biological components of the *Ixora coccinea* plant is flavonoids. HPLC was used in this investigation to quantitatively analyze the flavonoid and determine how much of it was present in the plant extract. For this research, methanolic leaf and flower extracts (1:10) were employed. The presence of flavonoids in the methanolic plant extracts of *Ixora coccinea* was confirmed using quercetin as a reference. Flavonoids were measured and the sample's peak area was computed. (10)

➢ *Biological Evaluation:*

▪ *Antioxidant Activity of Ixora Coccinea Plant Extract by DPPH Method:*

Antioxidants are among the many dietary phytonutrients found in functional foods, which have gained popularity when it was discovered that free radicals can be utilized to identify diseases. When analyzing natural antioxidants, determining their antioxidant activity is one of the most crucial tasks. DPPH (2,2diphenyl -1-picrylhydrazyl) and antioxidants from the extract react in a novel TLC spot method to assess the overall antioxidant activity of the plant extract.

The following formula was used to determine the free radical scavenging activity (FRSA) (% antiradical activity). (8) **% of antibacterial activity= (Absorbance of control − Absorbance of sample) x100 Absorbance of control**

➢ *Antibacterial Activity (Disc Diffusion Method)*

The disc diffusion method was used to test the polyphenol rich fraction's antibacterial properties. Mueller Hinton agar plates were used to cultivate the bacteria overnight. Five colonies were then suspended in five ml of sterile saline (0.9%), and the bacterial population in the suspension was brought down to about ~3x108CFU/ml (11). Each disc received a varying concentration of the polyphenol-rich fraction from *Ixora coccinea* flowers, while the control disc just got 7% ethanol. After the plates were incubated for 24 hours at 37°C, the inhibition zone was measured and computed. The tests were run three times in duplicate. The Zones of growth inhibition around the discs were measured in order to record the results (mean value, n=3) (9).

III. RESULTS

➢ *Qualitative Phytochemical Analysis*

Through screening, each extract's main phytoconstituents were discovered. Phytochemical screening results indicate that the methanolic extract contains higher levels of flavonoids, phenolic compounds, and other components. The phytoconstituents present in each extract were listed in Table 1. Additional therapeutic benefits may result from particular combinations of Phytochemical, or secondary metabolites, present in plant extracts.

➢ *Chromatographic Analysis*

- *Thin Layer Chromatography:*

For further research, the polyphenol-rich fraction from *Ixora coccinea* flowers put on pre-coated TLC plates and created using a solvent solution consisting of petroleum ether, chloroform, and methanol in a ratio of 1:0.5:0.1 was effective in extracting the antidiabetic chemical. The retention factor (Rf) is calculated. The formed plate was examined at UV wavelengths of 240 and 360 nm (Table 2) & (Figure no 5).

- *High Performance Liquid Chromatography:*

When compared to the standard quercetin, the HPLC examination of the leaf and flower revealed a peak for flavonoids. They computed the area under the peak. As illustrated in Figures no. 6, 7, and 8, the concentration of the leaf extract is 2.815 mg/ml and that of the floral extract is 3.171 mg/ml.

➢ *Biological Evaluation:*

- *Antioxidant Activity of Ixora Coccinea Plant Extract by DDPH Method:*

The DPPH technique was used to measure the methanolic leaf extract of *Ixoracoccinea's* total free radical scavenging activity. IC_{50} and the percentage of free radical

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scavenging activity inhibition were computed. Both conventional ascorbic acid and methanolic leaf extract have inhibitory percentages that range from 67.94% to 80.47% and 6.38% to 61.55%, respectively. The outcomes are displayed in Table no 3. For both methanolic leaf extract and the standard ascorbic acid, a graph showing the percentage of absorbance versus antioxidant concentration is plotted (Graph no 1). As the concentration rises, so does the absorption percentage.

- *Antibacterial Activity (Disc Diffusion Method):*

The antibacterial properties of the polyphenol-rich fraction from *i. Coccinea* flowers were tested against the microorganism's *pseudomonas klebsiellapneumoniae*, *staphylococcus aureus*, and *escherichia coli*. By calculating disc diffusion values, the extracts' antibacterial efficacy was evaluated (table 4). The polyphenol-rich fraction from *i. Coccinea* flowers was found to have a strong inhibitory effect on *s. Aureus* and *e. Coli*. The portion that was rich in polyphenols was more effective than *s. Aureus*. Among the various ways that phenolic compounds can function as antimicrobial agents are through the death of cells and the reduction of pathogenicity. Experimental design: All the experiments will be carried out in duplicate. For optimization, OFAT (one factor at a time) will be followed.

Table 2 Phytochemical screening of aqueous extract from flower of *Ixora coccinea*

Sl. No.	Phytochemical Constituents	Observation	Aqueous extract of flower of *Ixora coccinea*
1	Alkaloids		
	Dragendorff's test	Orange/red precipitate	+
	Mayers test	Yellow or white precipitate	+
2.	Flavonoids		
	Alkalai Reagent	Intense yellow colour	+
	Lead acetate test	Yellow Precipitate formed	+
3.	Glycosides		
	Keller-Killiani test	Reddish brown colourring formed	-
4.	Tannin-$FeCl_3$test	Blue black coloration	-
5.	Saponins		
	Frothingtest	Foam	+
6.	Terpenoids		
	Salkowskitest	Dark reddish brown colo rinterface	-
7.	Polyphenols		
	Ferrozine test	Raddish blue	+

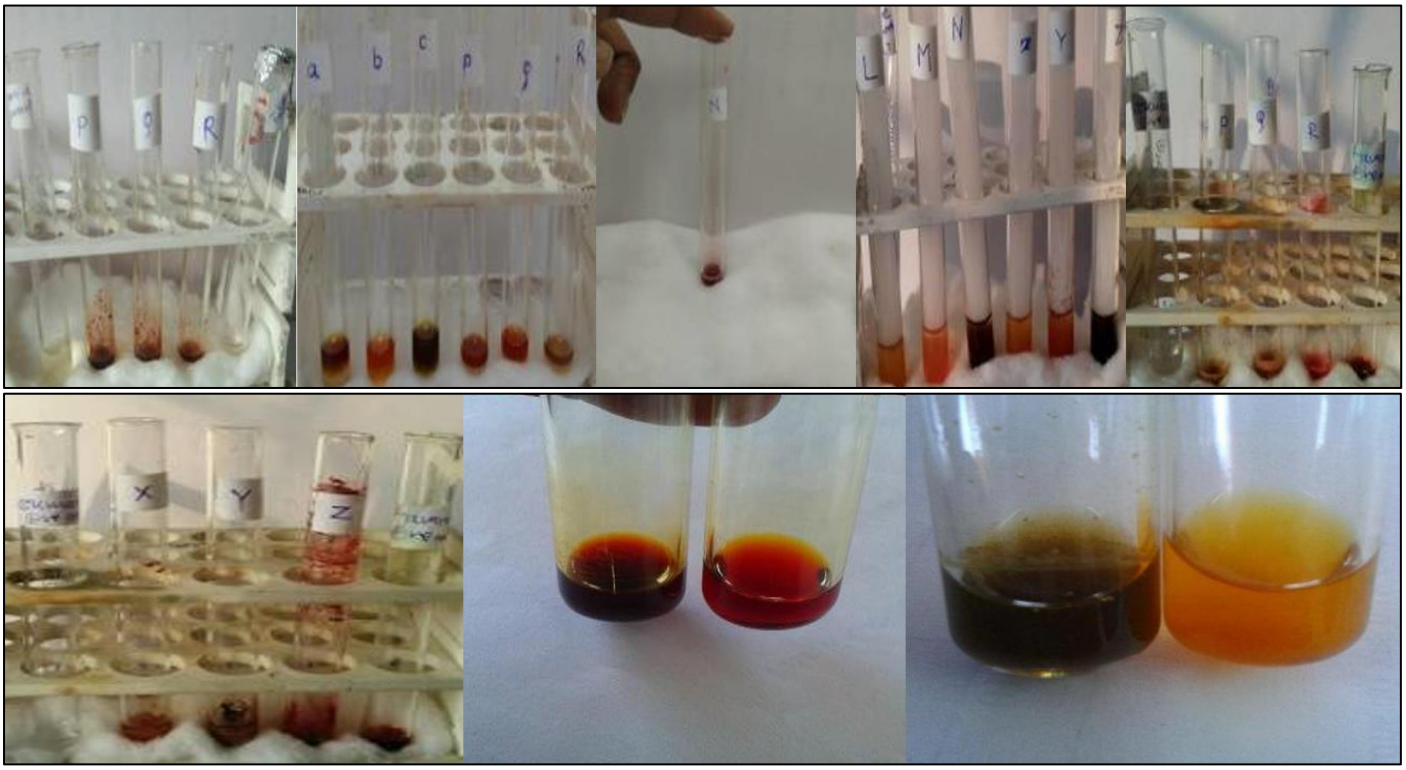

Fig 4 Qualitative analysis of phytochemical analysis such as flavonoid, tannin, terpenoids, Glycosides, alkaloids, polyphenols, and saponin tests are shows.

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Table 3 TLC Rf Value of polyphenol rich fraction from flower of *Ixoracoccinea*

S. No	UV240 nmRf value	UV360 nmRf value
1	-	0.86
2	0.67	0.67
3	0.48	0.48
4	0.27	0.27

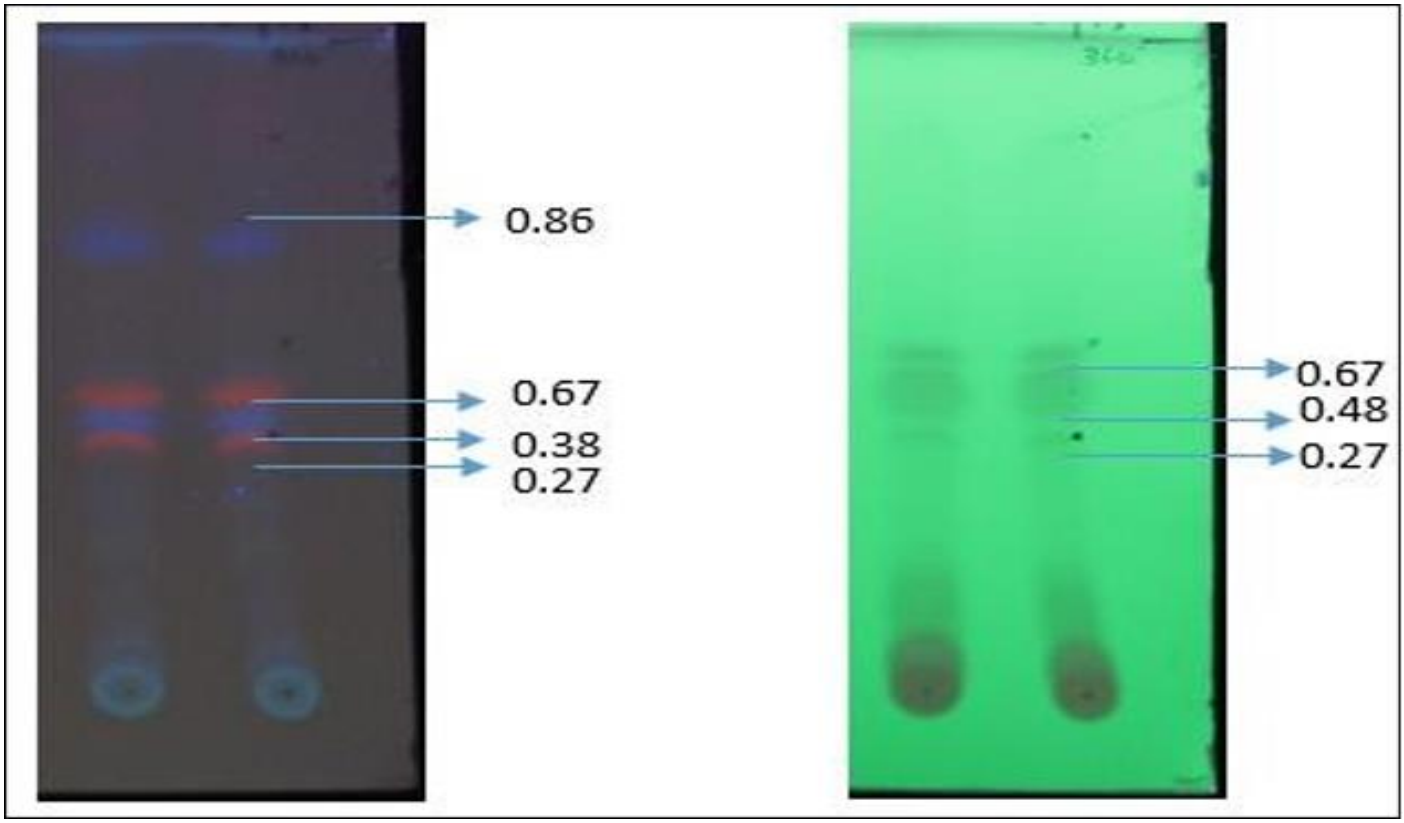

Fig 5 TLC plate viewed UV Light

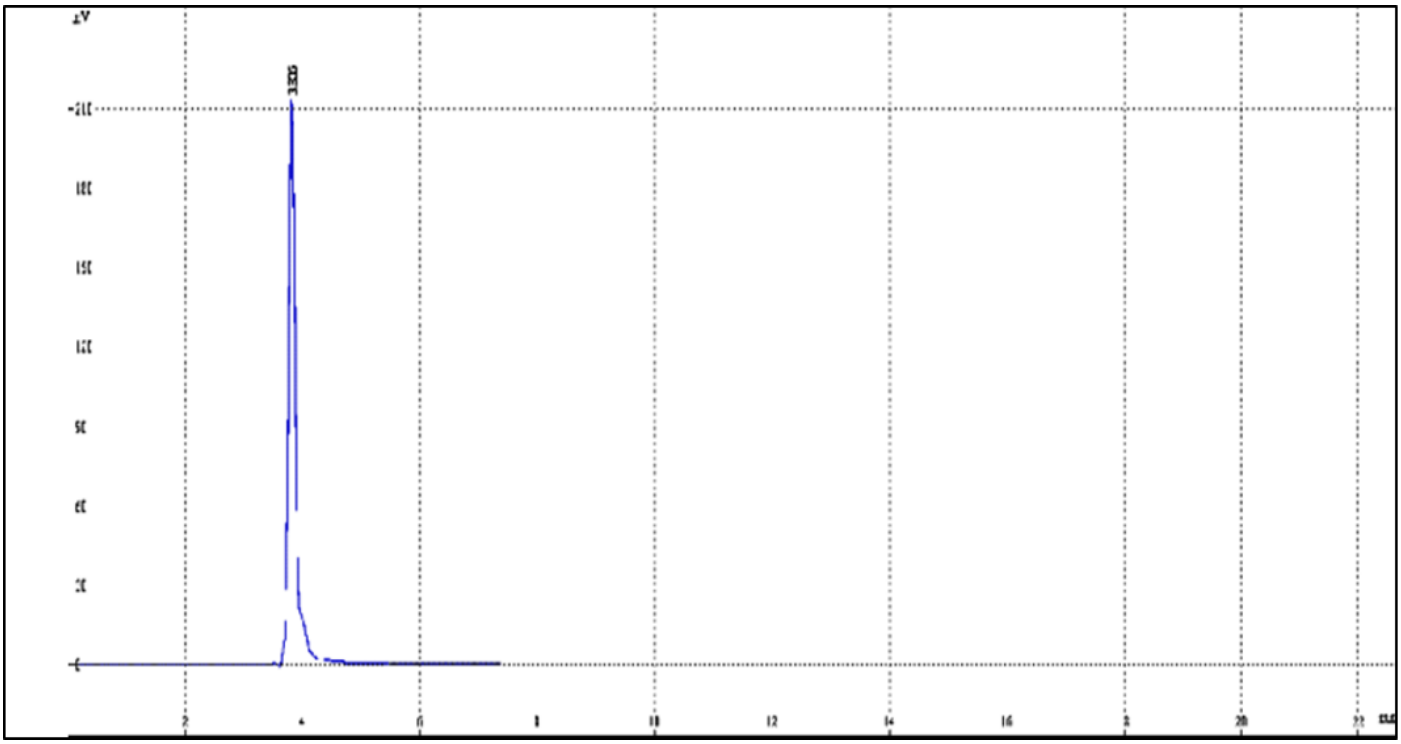

Fig 6 Chromatogram of Standard (Quercetin)

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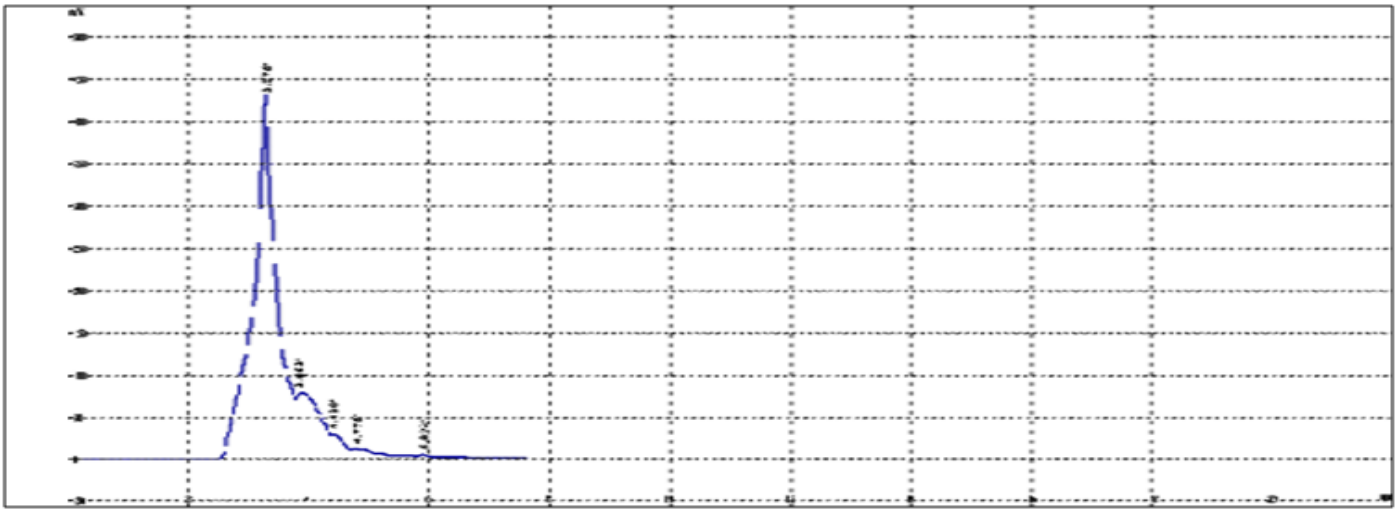

Fig 7 Chromatogram of Leaf Extract

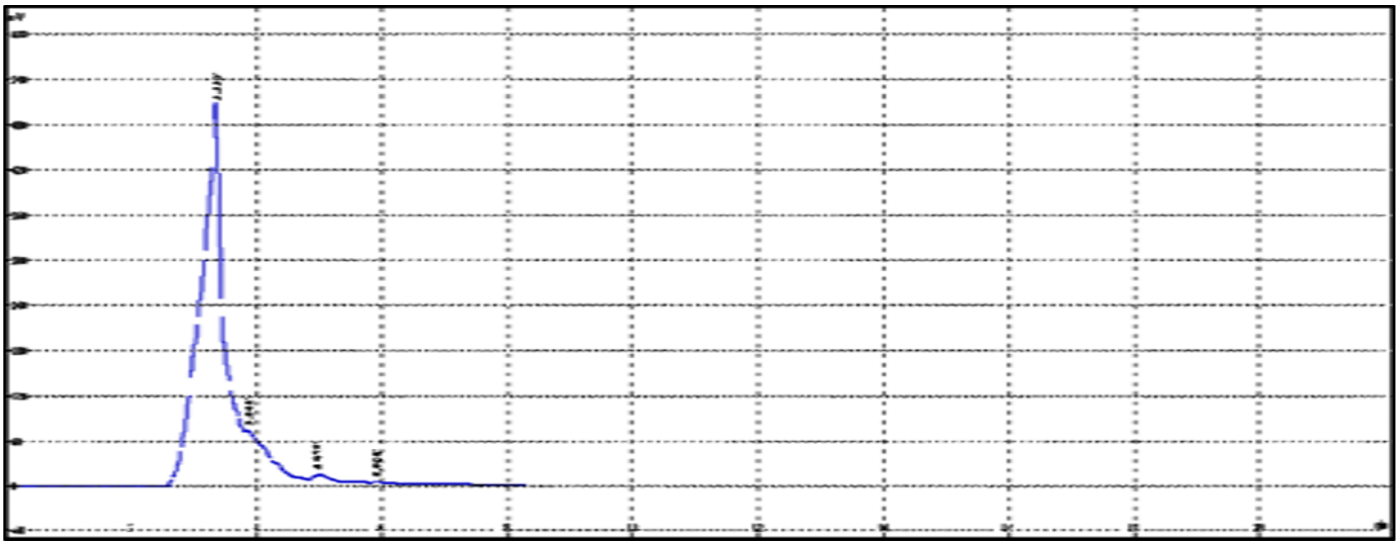

Fig 8 Chromatogram of Flower Extract

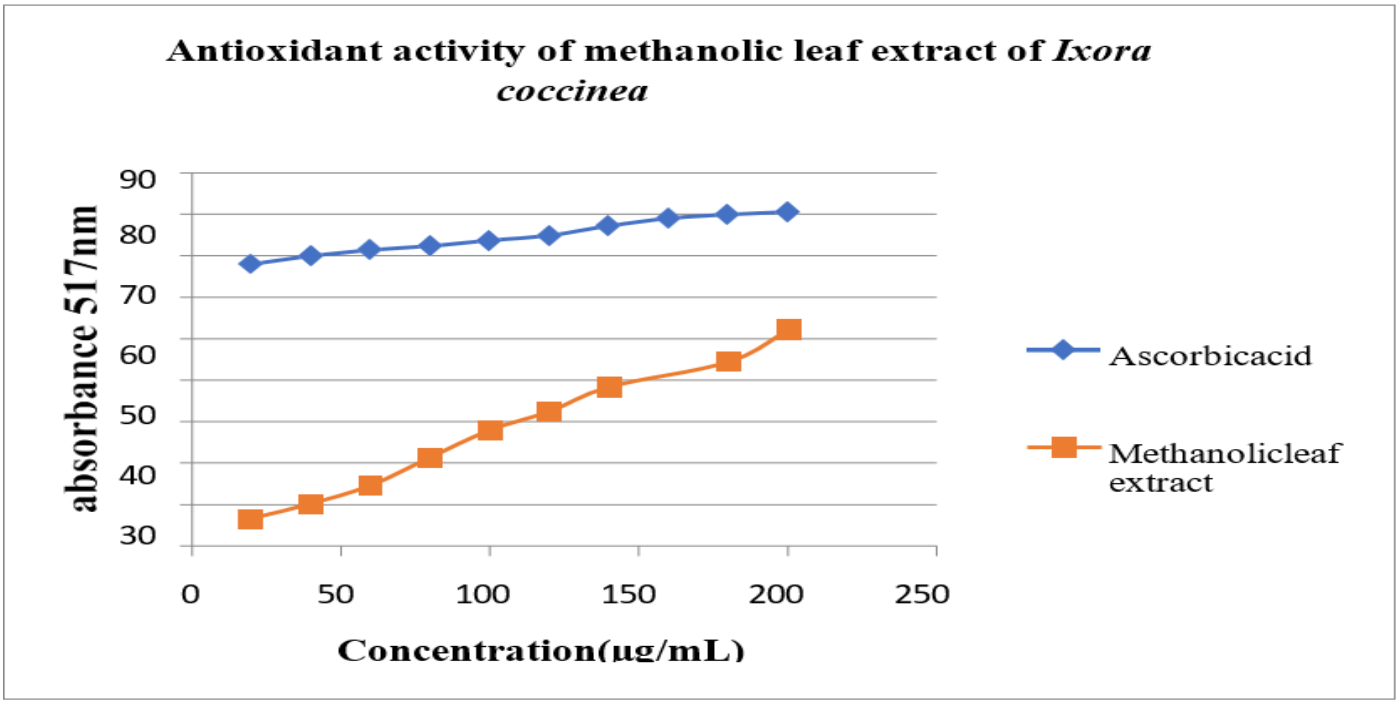

Fig 9 Antioxidant activity of methanolic leaf extract of Ixora coccinea L. by DPPH radical scavenging method

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Table 4 Antioxidant activity of methanolic leaf extract of Ixora coccinea L. by DPPH radical scavenging method

Concentration (µg/ml)	Standard Ascorbic acid	Methanolic extract (% inhibition)
20	67.94	6.38
40	69.9	9.99
60	71.33	14.41
80	72.31	21.19
100	73.63	27.85
120	74.84	32.38
40	77.13	38.33
160	78.92	44.4
180	79.83	52.21
200	80.47	61.55

Table 5 The antibacterial activity of the polyphenol rich fraction form flowers of *I. coccinea* by disc diffusion method.

Pathogenic organism	Different concentrations Crude extract (µl/ml)			
	25 µl/ml	50 µl/ml	75 µl/ml	100µl/ ml
Staphylococcusaureus	9.3±0.2	11.3±1.2	14.1±0.4	16.3±1.3
Escherichiacoli	8.2±2.5	10.3±1.5	13.6±1.3	15.4±0.5
Pseudomonasaeruginosa	7.8±1.3	8.6±0.8	11.3±1.6	13.7±1.8
Enterococcusfaecalis	6.7±0.9	7.9±2.4	10.7±1.4	12.3±2.3

*The 6.0 mm well size was measured using a caliper as part of the inhibitory zone size. The mean values were noted after each assay was replicated.

IV. DISCUSSION

The current study concentrated on the thorough phytochemical profiling and biological assessment of *Ixora coccinea* floral extract. Key secondary metabolites, including flavonoids, alkaloids, tannins, phenolic, and terpenoids, were detected by preliminary phytochemical screening. These substances may greatly enhance the plant's pharmacological potential and are well-known for their strong biological effects. Chromatographic methods such as High-Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) validated the variety of phytoconstituents in the extract. The emergence of discrete peaks and bands suggests the existence of several bioactive substances, some of which might be identified as antimicrobials and recognized antioxidants from earlier research on *Ixora* species.

The antioxidant activity assessed through DPPH free radical scavenging assay demonstrated strong radical scavenging potential, suggesting that the extract is rich in phenolic and flavonoid content. These compounds are effective in neutralizing free radicals, thereby reducing oxidative stress, which is associated with various chronic diseases. The antibacterial activity showed promising inhibitory effects against both Gram-positive and Gram-negative bacteria, particularly *Staphylococcus aureus* and *Escherichia coli.* The historic use of *Ixora coccinea* flowers in folk medicine to treat wounds and infections is supported by this. The combined activity of several phytochemicals, particularly alkaloids and flavonoids may be responsible for the antimicrobial results. Overall, the results bolster the ethno medical value of *Ixora coccinea* flowers and emphasize their potential as a natural source of antimicrobials and antioxidants. For pharmaceutical development, more research on chemical separation and mechanism of action will be crucial.

V. SUMMARY AND CONCLUSION:

➢ *Summary*

The complex phytochemical profile and intriguing biological qualities of the widely distributed ornamental plant *Ixora coccinea* have attracted a lot of scientific attention. The presence of a variety of bioactive substances, such as flavonoids, phenolics, alkaloids, tannins, and saponins, which are known to contribute to therapeutic benefits, was discovered through phytochemical screening of its flower extract. Identification and characterization of these elements have been made possible by chromatographic techniques like High-Performance Liquid Chromatography (HPLC), and Thin Layer Chromatography (TLC). Phenolic and flavonoid content may be measured using HPLC, and complex secondary metabolites can be structurally understood with MS. Due in significant part to the high phenolic content, antioxidant activity measured by DPPH and ABTS assays exhibits robust radical scavenging ability.

Additionally, the floral extract has demonstrated strong inhibitory effects, the extract exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria.in antibacterial investigations employing disc diffusion and MIC techniques, indicating potential for antimicrobial applications. All things considered, these results demonstrate *Ixora coccinea's* potential as a natural source of antioxidants and antibacterial and lend credence to its long-standing use in herbal therapy.

➢ *Conclusion*

Ixora coccinea flower extract's rich phytochemical makeup, which includes flavonoids, phenolic, alkaloids, and glycosides, has been confirmed by thorough analysis. Numerous bioactive components were identified by chromatographic methods like TLC and HPLC, while mass spectrometry offered molecular insights and structural

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validation. Due to its high phenolic content, the extract demonstrated significant antioxidant activity, indicating its potential to scavenge free radicals and mitigate oxidative stress.

Additionally, it had strong inhibitory effects against both Gram-positive and Gram-negative bacteria in antibacterial testing, indicating that it might be used in herbal antimicrobial compositions. These results support *Ixora coccinea's* further development as a natural medicinal agent and validate its traditional applications.

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