International Journal of Scientific Research in Science and Technology
Available online at : www.ijsrst.com
Print ISSN: 2395-6011 | Online ISSN: 2395-602X
doi : https://doi.org/10.32628/IJSRST251222599

# Comparative Phytochemical Evaluation and Dyeing Potential of Ixora Coccinea Flowers under Saline and Non-Saline Environment

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ARTICLEINFO

**Article History:**
Accepted : 29 March 2025
Published: 02 April 2025

**Publication Issue :**
Volume 12, Issue 2
March-April-2025

**Page Number :**
582-590

ABSTRACT

Abiotic environmental stresses trigger complex and adaptable reactions in plants. The salinity stress induced modifications were studied in the flowers of Ixora coccinea sourced from Bhavnagar with elevated soil salinity and for non-saline sample Ahmedabad area was chosen for flower collection. Comparative qualitative phytochemical analysis, total phenolic content and total flavonoid content was performed which indicated higher phenolic and flavonoid contents in Bhavnagar (saline) sample. Furthermore, the detection of brassinosteroids (BRs) in the saline-exposed sample suggests an adaptive response to salinity stress. Evaluation of the dyeing potential and durability of floral dyes, extracted with four solvents from both saline and non-saline samples, showed warmer colour development in non-saline extracts and cooler colour development in saline extracts. The study provides insights which may aid in the production of improved herbal dyes from I. coccinea.

**Keywords:** Salinity stress, Ixora coccinea, Flowers, Phytochemical analysis, Phenolics, Flavonoids, Floral dye

## I. INTRODUCTION

*Ixora coccinea* L., a plant in the Rubiaceae family, is recognized as 'Jungle geranium' or 'Vetchi' within Ayurveda. It is an evergreen flowering shrub native to the tropical regions of Asia. Traditionally, Ayurvedic and folk medicine have employed *I. coccinea* flowers to treat diverse ailments, including leukorrhea, dysentery, dysmenorrhea, hemoptysis, hypertension, menstrual irregularities, sprains, bronchitis, fever, sores, chronic ulcers, scabies, and skin conditions (Balinga and Kurian, 2012). Various pharmacological studies have demonstrated wide range of therapeutic properties of *Ixora* plant including antimicrobial, anti-inflammatory, antidiarrheal, antitumor, and antioxidant effects (Guddi *et al.*, 2022); which can be attributed to the presence of a wide range of phytochemicals, including flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins, isocatechins, alkaloids, tannins, saponins, and triterpenoids (Elumalai *et al.*, 2012). A wide array of colour pigments has also been reported such as

anthocyanin, betalains, cyanidin, carotenoids, xanthophyll, phycoerythrin, and phycocyanin (Elumalai *et al*., 2012; Pujari *et al*., 2022).

Colour is an integral part of the natural world, enriches human experience, making life more visually stimulating and intriguing. Over the centuries, plant pigments have been used to provide colours for various applications, including cave paintings, architectural works, and fabric dyeing. The historical use of natural dyes for textiles is now being expanded, with increased interest in plant-derived colours for textiles, food, pharmaceutical, and cosmetic products. The rising demand for plant-based dyes, especially in textiles, stems from consumers' preference for natural compounds over synthetic chemicals (Patil and Datar, 2015). *Ixora* flowers can yield an attractive pinkish-red dye, derived from their anthocyanin pigments (Patil and Datar, 2015). Anthocyanins are pigments belonging to the flavonoid family that are soluble in water and produced through the shikimic acid pathway (Chalker-Scott, 1999). Researchers have documented the use of *Ixora* flower extract in cloth dyeing, noting the requirement for mordants to ensure colour fastness (Patil and Datar, 2015). Salinity, an abiotic stress, not only alters the morphological and physiological characteristics of plants but also significantly influences pigment production often leading to changes in coloration (Hashemi and Shahani, 2019). It has been consistently reported that environmental stresses induce a notable elevation in total phenol and anthocyanin concentrations (Chalker-Scott, 1999; Parida and Das, 2005). The present study focusses to deliberate the impact of salinity on phytochemicals of flowers of *I. coccinea* and variations observed in the colours of the dye extracted from the flowers collected from non-saline and saline environmental conditions.

## II. MATERIALS AND METHODS

### 2.1 Collection and Authentication of Plant Material

Fresh flowers of *I. coccinea* were collected from Ahmedabad – (non-saline area, 23.0354° N, 72.5444° E) and Bhavnagar – (saline area, 21.7538° N, 72.1834° E); and authenticated by botanical experts. The flowers were thoroughly cleaned and shade-dried for 10 days. The dried flowers were comminuted to a fine powder, yielding approximately 30 g of material individually from both the samples.

### 2.2 Qualitative phytochemical analysis

#### 2.2.1 Preparation of flower extract

Extraction of the dried flowers was carried out using 30 gm of fine powder from both the samples, dissolving them into 300 mL of hydroalcoholic solvent prepared using methanol and distilled water (6:4). It was kept for maceration for 48 hours followed by filtration using Whatman filter paper no.1. The filtrate was then poured to perti dishes and dried for 3 days resulting into crude. Crude was further dissolved into solvent to prepare 5 mg/mL of flower extract.

#### 2.2.2 Preliminary phytochemical screening

The hydroalcoholic extracts underwent systematic qualitative phytochemical analysis for various constituents (Yadav and Agarwala, 2011; Pandey and Tripathi, 2013; Ayu *et al*., 2018).

- Alkaloids: Using Mayer's and Wagner's test.
- Anthocyanins: Using HCl and NaOH.
- Carbohydrates: Using Fehling's and Benedict's test.
- Flavonoids: Through alkaline reagent (NaOH) and lead acetate tests.
- Proteins: Using Biuret test (General test) and Million's test.
- Quinones: Using 2% NaOH.
- Saponins: Using Foam test.
- Steroids: Using Salkowski reaction.
- Terpenoids: Using modified Salkowski test.
- Tests for tannins and phenolic compounds: Using Lead acetate solution and 3% $FeCl_3$ solution.

### 2.3 Quantitative analysis for phenols and flavonoids

#### 2.3.1 Total phenolic content

Total phenolic content was determined using Folin-Ciocalteu (FC) reagent. The plant extract (0.5 mL) was mixed with 0.5 mL of FC reagent (1:10 diluted with distilled water) followed by addition of 2 mL of 20% $Na_2CO_3$. The mixture was then incubated further at 22°C for 90 min and the absorbance was measured at 765 nm. The total phenolic content (mg/mL) was calculated using gallic acid as standard and was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried sample weight (mg GAE/g DW) (Sankhalkar and Vernekar, 2016).

#### 2.3.2 Total flavonoid content

The total flavonoid content (mg/mL) was determined using aluminium chloride ($AlCl_3$) method. The assay mixture consisting of 0.5 mL of the plant extract, 0.5 mL of 30% methanol and 0.3 mL of 5% $NaNO_2$ was incubated for 5 min at 25°C This was followed by addition of 0.3 mL of 10% $AlCl_3$ immediately. Then 2 mL of 1 M NaOH was then added to the reaction mixture, and the absorbance was measured at 510 nm. Quercetin was used as a standard and the results are expressed as milligrams quercetin of equivalents per gram of dry sample weight (mg QE/g DW). (Sankhalkar and Vernekar, 2016).

### 2.4 Extraction of dye from I. coccinea flowers

#### 2.4.1 Source and substrate

A fine powder was obtained after washing and drying *I. coccinea* flowers which were collected from Bhavnagar – (saline area, 21.7538° N, 72.1834° E) and Ahmedabad – (non-saline area, 23.0354° N, 72.5444° E). For this investigation, 15x15 cm samples of 100% soft cotton fabric were employed as the base material.

#### 2.4.2 Chemicals

The different chemicals such as Distilled Water, Copper Sulphate ($CuSO_4$), Sodium Hydroxide (NaOH), Acetic Acid ($CH_3COOH$), 98% Methanol and 95% Ethanol; were used in this investigation.

#### 2.4.3 Extraction methods

(a) *Aqueous extraction method*

In this method, 10 gm of flower petal powder was boiled in 100 mL distilled water at 100 °C for 30 minutes. The extraction solvent was then filtered by muslin cloth.

(b) *Alkaline extraction method*

In this method, 10 gm of flower petal powder was boiled in 75 mL distilled water with 25 mL of 2% NaOH for 30 minutes at 100 °C temperature. The decolourized powder was then taken out and extraction solvent was filtered by muslin cloth.

(c) *Acidic extraction method*

In this method, 10 gm of flower petal powder was boiled in 75 mL distilled water with 25 mL of acetic acid for 30 minutes at 100 °C temperature. Following removal of the decolorized powder, the extraction solvent underwent filtration using muslin cloth.

(d) *Alcoholic extraction method*

In this method, 10 gm of flower petal powder was boiled in 80 mL methanol with 20 mL of ethanol for 30 minutes at 100 °C temperature. Muslin cloth was used to filter the extraction solvent.

#### 2.4.4 Scouring of fabric

Prior to the dyeing procedure, the cotton cloths underwent a 10-minute boil in a 10% sodium hydroxide (NaOH) solution to eliminate starch and other impurities. Subsequently, the NaOH-treated cloths were washed extensively with cold distilled water (Patil *et al.*, 2016).

#### 2.4.5 Dyeing and Mordanting

Natural dyes typically require mordants, a mordant is an organic reagent (mostly metallic salts) which supports to form a complex among dye and fabric to achieve lasting colour (Narmatha and Sagaya Giri, 2020). Copper sulphate ($CuSO_4$) was used as a mordant. The scoured cotton cloths were treated with mordanting solution M: L (material to liquor) ratio as 1:20 at temperature range of 70-80 °C. Each extracted dye was used to dye fabric at the same temperature for 30 minutes, followed by washing with cold water, squeezing, and air-drying.

### 2.4.6 Evaluation of dyed samples

The colours developed on the cotton fabric through extracted dye were identified using the ISCC-NBS colour system, the colour variation due to environmental factor was noted. After dyeing, fabrics were subjected to colour fastness test to assess their colour durability against light, washing and rubbing according to ISO 105 and AATCC standards.

## III.RESULTS

### 3.1 Qualitative phytochemical analysis

Our results with various phytochemical tests are shown in Table 1. The results shown that both the samples containing flowers of *I. coccinea* namely IC-A (non-saline) and IC-B (saline) are rich in alkaloids, anthocyanins, flavonoids, phenols, proteins, quinones, saponins, tannins and terpenoids. Steroids were found to be present only in saline sample of *I. coccinea.*

**Table 1.** Qualitative phytochemical analysis of two screened plant samples of *I. coccinea* of non-saline and saline environment * + : present; – : absent

| Phytoconstituents | Test | IC-A (non-saline sample) | IC-B (saline sample) |
|---|---|---|---|
| Alkaloids | Mayer's Test | + | + |
| | Wagner's Test | + | + |
| Anthocyanins | | + | + |
| Carbohydrates | Fehling's Test | - | - |
| | Benedict's Test | - | - |
| Flavonoids | Alkaline reagent Test | + | + |
| | Lead acetate Test | + | + |
| Phenols | | + | + |
| Proteins | Biuret Test | - | - |
| | Million's Test | - | - |
| Quinones | | + | + |
| Saponins | | - | - |
| Steroids | Salkowski Test | - | + |
| Tannins | | + | + |
| Terpenoids | | + | + |

### 3.2 Total phenolic content

The results of total phenolic content in IC-A (non-saline) and IC-B (saline) are shown in Table 2. Our results with hydroalcoholic flower extracts for saline samples are 2.83% higher than non-saline sample.

**Table 2.** Total phenolic content in the flower samples of *I. coccinea* from non-saline and saline environment

| Plant sample | Flower (mg/mL) |
|---|---|
| IC-A (non-saline) | 84.13 ± 0.23* |
| IC-B (saline) | 86.55 ± 2.49* |

*The values are mean of three experiments ± SD. SD: Standard deviation

### 3.3 Total flavonoid content

The results of total flavonoid content in IC-A (non-saline) and IC-B (saline) are shown in Table 3. Our results with hydroalcoholic flower extracts for saline samples are 26.48% higher than non-saline sample.

**Table 3.** Total flavonoid content in the flower samples of *I. coccinea* from non-saline and saline environment

| Plant sample | Flower (mg/mL) |
|---|---|
| IC-A (non-saline) | 2.13 ± 0.06* |
| IC-B (saline) | 2.78 ± 0.03* |

*The values are mean of three experiments ± SD. SD: Standard deviation

### 3.4 Extraction of dyes from I. coccinea flowers

The colours developed on the cotton fabric through extracted dye were identified using the ISCC-NBS colour system are shown in Figures 1(a-d) and 2(a-d). The Rating of fastness properties of dye are given in the Table 4.

**Table 4.** The colour fastness to washing, light, rubbing of dyed cotton samples with four different extracts

| Sample | Washing fastness (Grade) | Light fastness (Grade) | Rubbing fastness (Grade) | |
|---|---|---|---|---|
| | | | Dry | Wet |
| IC-A (non-saline) | 4 | 4 | 4–5 | 4 |
| IC-B(saline) | 4 | 4 | 4–5 | 4 |

**Figure 1. Colours developed on cotton fabric from IC-A (non-saline) sample dye extraction using different solvents**

**Figure 2. Colours developed on cotton fabric from IC-B (saline) sample dye extraction using different solvents**


## IV. DISCUSSION

The successful isolation and characterization of about 54 phytochemicals from *I. coccinea* and its constituent parts demonstrate the plant's rich chemical composition (Sumathy *et al.*, 2011). Representing 99.97% of the total detected phytochemical components, the flower extract revealed a composition dominated by triterpenoids (62.60%) and monoterpenoids (31.73%), with smaller percentages of esters (2.29%) and sesquiterpenes (3.35%) (Obuzor and Nwakanma, 2011). Phytochemical analysis indicated the presence of a wide range of bioactive compounds in investigated samples; multiple tests confirmed the presence of alkaloids, which holds particular significance due to their frequent association with therapeutic properties. Consistent with earlier studies on *I. coccinea*, our analysis revealed the presence of flavonoids and terpenoids (Donth *et al.*, 2015).

Abiotic environmental stresses trigger a variety of intricate responses in plants, reflecting their inherent plasticity. These responses, encompassing morphological, cellular, anatomical, and physiological modifications, are significantly mediated by phytohormones, which facilitate plant acclimatization (Fahad *et al.*, 2014). Bhavnagar's proximity to the Gulf of Khambhat subjects it to considerable saltwater impact, resulting in heightened soil salinity in the area (Pandya *et al.*, 2022). Saline stress in plants appears to trigger stress-protective responses, evidenced by the presence of steroids namely brassinosteroids (BRs) in IC-B (saline) sample which are proven to be vital for growth, development, and environmental adaptation (Planas-Riverola *et al.*, 2019). Responses to environmental stresses are largely governed by the abscisic acid (ABA) signalling pathway (Yoshida *et al.*, 2014). Beyond this established role of abscisic acid (ABA) in environmental stress adaptation, brassinosteroids (BRs) are implicated in the fine-

tuning of growth-defence homeostasis, exerting influence via both crosstalk with the ABA pathway and through distinct, parallel signalling routes (Wang *et al.*, 2020; Lima and Lobato, 2017).

The genus *Ixora* is a rich source of flavonoids, a highly prevalent group of phenolic compounds, from which approximately 17 distinct flavonoids, including flavones, flavanols, flavan-3-ols (catechin), anthocyanins, and pro-anthocyanidins, have been successfully isolated and characterized (Nadeem *et al.*, 2024). The phenolic and flavonoid contents were significantly higher in saline samples of *I. coccinea* which indicates the possible involvement of secondary metabolites in salt tolerance. Salt-induced oxidative stress in plants leads to an enhancement of the flavonoid biosynthetic pathway, causing an increase in flavonoid levels as a strategy for coping with stress (Wahid and Ghazanfar, 2006). The non-enzymatic antioxidant properties of phenolics, which encompass flavonoids, are essential for the mitigation of plant stress via the effective quenching of free radicals and the subsequent reduction of reactive oxygen species (ROS) (Michalak, 2006; Bartwal *et al.*, 2012).

Extracts of *I. coccinea* flowers, prepared using various solvents, demonstrated significant dyeing efficacy on cotton fabric, evidenced by colour fastness ratings of 4 to 5, indicating notable durability. Cotton fabric dyed with extract from the non-saline sample exhibited a tendency towards warmer hues, despite a comparatively lower flavonoid concentration. The observed cooler chromatic properties of the saline sample extract were contradictory to its elevated flavonoid content, leading to a need for a more thorough examination. Due to their high pH sensitivity, anthocyanin pigmentation in plants is affected by salinity stress, which may induce changes in cellular pH, leading to observable colour variations from red to blue (Khoo *et al.*, 2017). The discrepancy of cooler colours with higher flavonoid content may be explained by salinity-driven changes in dye-fibre interactions or pH-induced modifications at cellular level.

## V. COCLUSIONS

The natural dyes are now trending all over the world due to rising awareness about toxicity hazards of the synthetic dyes. This study concludes that *I. coccinea* flowers exposed to saline environments of Bhavnagar accumulate significantly higher concentrations of phenolic and flavonoid compounds compared to those from non-saline environments of Ahmedabad, suggesting that salinity stress induces the production of these compounds as an adaptive mechanism. Furthermore, both saline and non-saline flower samples were found to contain substantial levels of flavonoids, phenolics, and tannins. In addition, steroids were present in saline sample indicating the environmental stress adaptation to salinity stress. Extracts from both flower samples exhibited considerable dyeing effectiveness on cotton, achieving colour fastness ratings between 4 and 5, signifying remarkable durability. The results prove the potential of *I. coccinea* as a source for herbal dyestuff extraction. However, to obtain the dyes with adequate fastness, methods for rigorous selection of the plant material and development of appropriate extraction processes are required.

## REFERENCES

[1]. Ado, A., Yahaya, H., Kwalli, A. A., & Abdulkadir, R. S. (2014). Dyeing of textiles with eco-friendly natural dyes: a review. International Journal of Environmental Monitoring and Protection, 1(5), 76-81.

[2]. Ahire, B. B. Natural Dye Extractions from Selected Plants, Extraction and Mordant Application to Dyeing Cotton Fabric.

[3]. Ayu, A. C., Ida, M., Moelyono, M., & Fakhriati, S. G. (2018). Total anthocyanin content and identification of anthocyanidin from

Plectranthus scutellarioides (L.) R. Br leaves. Res. J. Chem. Environ, 22, 11-17.

[4]. Baliga, M. S., & Kurian, P. J. (2012). Ixora coccinea Linn.: Traditional uses, phytochemistry and pharmacology. Chinese journal of integrative medicine, 18, 72-79.

[5]. Bartwal, A., Mall, R., Lohani, P., Guru, S. K., & Arora, S. (2013). Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. Journal of plant growth regulation, 32, 216-232.

[6]. Chalker-Scott, L. (1999). Environmental significance of anthocyanins in plant stress responses. Photochemistry and photobiology, 70(1), 1-9.

[7]. Elumalai, A., Eswaraiah, C., Venkatesh, Y., & Narendar, C. (2012). Phytochemical and pharmacological profile Of Ixora coccinea Linn. International Journal of Pharmacy & life sciences, 3(3).

[8]. Fahad, S., Hussain, S., Matloob, A., Khan, F. A., Khaliq, A., Saud, S., ... & Huang, J. (2015). Phytohormones and plant responses to salinity stress: a review. Plant growth regulation, 75, 391-404.

[9]. Guddi, K., Sur, S., & Sarkar, A. (2024). Analysis of floral biomass utilization feasibility of Ixora coccinea for its cost-effective application as a natural colorant. Biomass Conversion and Biorefinery, 14(14), 15661-15675.

[10]. Hashemi, A., & Shahani, A. (2019). Effects of salt stress on the morphological characteristics, total phenol and total anthocyanin contents of Roselle (Hibiscus sabdariffa L.). Plant Physiology Reports, 24, 210-214.

[11]. Jothi, D. (2008). Extraction of natural dyes from African marigold flower (Tagetes ereecta L) for textile coloration. Autex Research Journal, 8(2), 49-53.

[12]. Lima, J. V., & Lobato, A. K. S. (2017). Brassinosteroids improve photosystem II efficiency, gas exchange, antioxidant enzymes and growth of cowpea plants exposed to water deficit. Physiology and Molecular Biology of Plants, 23, 59-72.

[13]. Michalak, A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish journal of environmental studies, 15(4).

[14]. Narmatha, P. R. Sagaya giri.(2020). Extraction of natural dye from Ixora coccinea Linn. flowers and evaluation of cosmetics as face powder dyeing using different chemical mordants. Asian Journal of Innovative Research, 5(1), 7-14.

[15]. Obuzor, G. U., & Nwakanma, G. U. (2011). Chemical composition of essential oil of ixora coccinea flower from Port Harcourt, Nigeria.

[16]. Pandey, A., & Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and phytochemistry, 2(5).

[17]. Parida, A. K., & Das, A. B. (2005). Salt tolerance and salinity effects on plants: a review. Ecotoxicology and environmental safety, 60(3), 324-349.

[18]. Patil, N. N., & Datar, A. G. (2016). Applications of natural dye from Ixora coccinea L. in the field of textiles and cosmetics. Coloration Technology, 132(1), 98-103.

[19]. Planas-Riverola, A., Gupta, A., Betegón-Putze, I., Bosch, N., Ibañes, M., & Caño-Delgado, A. I. (2019). Brassinosteroid signaling in plant development and adaptation to stress. Development, 146(5).

[20]. PUJARI, V., SAWANT, R., SHIVATHAYA, N., SURVE, R., SUNAGAR, N., SAWANT, V., & PATIL, S. (2022). Formulation And Evaluation Of Lipstick Using Ixora Coccinea Flower Extract As A Natural Coloring Agent. Innovare J Ay Sci, 10(1), 1-5.

[21]. Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total

flavonoid contents of whole plant extracts Torilis leptophylla L. BMC complementary and alternative medicine, 12, 1-12.

[22]. Saline stress in plants appears to trigger stress-protective responses, evidenced by the presence of steroids, including brassinosteroids (BRs), which are vital for growth, development, and environmental adaptation

[23]. Sankhalkar, S., & Vernekar, V. (2016). Quantitative and qualitative analysis of phenolic and flavonoid content in Moringa oleifera Lam and Ocimum tenuiflorum L. Pharmacognosy research, 8(1), 16.

[24]. Siva, R. (2007). Status of natural dyes and dye-yielding plants in India. Current science, 916-925.

[25]. Sumathy, H., Sangeetha, J., & Vijayalakshmi, K. (2011). Chromatographic fingerprint analysis of Ixora coccinea methanolic flower extract.

[26]. Trivellini, A., Gordillo, B., Rodríguez-Pulido, F. J., Borghesi, E., Ferrante, A., Vernieri, P., ... & Heredia, F. J. (2014). Effect of salt stress in the regulation of anthocyanins and color of Hibiscus flowers by digital image analysis. Journal of agricultural and food chemistry, 62(29), 6966-6974.

[27]. Trotman, E. R. (1975). Dyeing and chemical technology of textile fibers, Charles Griffin Co. Ltd., London.

[28]. Wahid, A., & Ghazanfar, A. (2006). Possible involvement of some secondary metabolites in salt tolerance of sugarcane. Journal of plant physiology, 163(7), 723-730.

[29]. Wang, Q., Yu, F., & Xie, Q. (2020). Balancing growth and adaptation to stress: Crosstalk between brassinosteroid and abscisic acid signaling. Plant, Cell & Environment, 43(10), 2325-2335.

[30]. Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. Journal of phytology, 3(12).

[31]. Ye, H., Liu, S., Tang, B., Chen, J., Xie, Z., Nolan, T. M., ... & Yin, Y. (2017). RD26 mediates crosstalk between drought and brassinosteroid signalling pathways. Nature Communications, 8(1), 14573.

[32]. Yoshida, T., Mogami, J., & Yamaguchi-Shinozaki, K. (2014). ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. Current opinion in plant biology, 21, 133-139.