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PHYTOCHEMICAL SCREENING AND IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF *IXORA COCCINEA* EXTRACT USING THE DPPH RADICAL SCAVENGING ASSAY

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ABSTRACT

Ixora coccinea is a medicinal plant traditionally used for various therapeutic applications and is considered a potential source of bioactive phytoconstituents. The present study was undertaken to prepare and evaluate an extract of *Ixora coccinea* with particular emphasis on its phytochemical composition and antioxidant activity. The plant material was collected, cleaned, dried, pulverized, and authenticated by the National Siddha Institute, Arumbakkam, Chennai. The powdered plant material was subjected to Soxhlet extraction using 70% ethanol for 6–8 hours. The concentrated extract obtained was 12.5 g from 100 g of powdered plant material, corresponding to a percentage yield of 12.5%. Preliminary phytochemical screening indicated the presence of various classes of phytoconstituents, including alkaloids, flavonoids, tannins, phenolic compounds, saponins, glycosides, terpenoids, and steroids. The antioxidant potential of the extract was evaluated by the DPPH free radical scavenging assay at concentrations ranging from 10 to 500 µg/ml, using ascorbic acid as the standard. The extract demonstrated concentration-dependent DPPH radical scavenging activity. The percentage inhibition increased from 38.08% at 10 µg/ml to 84.15% at 500 µg/ml, while ascorbic acid showed 91.63% inhibition. The IC_{50} value of the tested extract was found to be

126.3 µg/ml, with an R^2 value of 0.9853, indicating a good concentration-response relationship. The findings suggest that the 70% ethanolic extract of *Ixora coccinea* possesses appreciable antioxidant activity, which may be attributed to its phytochemical constituents.

KEYWORDS: *Ixora coccinea*, phytochemical screening, ethanolic extract, antioxidant activity, DPPH, free radical scavenging, IC_{50}, flavonoids, phenolic compounds.

1. INTRODUCTION

The plants are obtained from the wild sources such as forest, plains, riverbanks etc. Obtaining herbs from wild source is my economical, less time consuming, Decreased cost of labour However it also offer various disadvantages such as quality of the plants cannot be Predicted due to various environmental changes. The plants will not be uniform in Their growth and yielding characteristics. Modern scientific techniques cannot be applied to increase the yield as well as Quality. If the plants are obtained continuously from wild source for prolonged periods a May lead to depletion of raw materials from the wild.

Plants and natural products were used by humankind over the years as food and medicines to cure and prevent diseases. It is very difficult to point out an exact time when the use of plants was started as medicine, the Carbon dating from ancient Babylon (Iraq) records that plants were cultivated as 60,000 years ago. Written material Medica of medicinal herbs go back approximately 5,000 years in India, China and Egypt and at least 2,500 years in Greece and Asia. It described well-established medicinal uses for plants such as laurel, caraway, and thyme at least 5,000 years ago. Egyptian people where well known to medicine before 2900 BC, these people used papyrus (pithy stem of a water plant for writing or painting) such as the Ebers papyrus(1500 BC) , the Edwin Smith papyrus (1600 BC), the Berlin papyrus (1200 BC), and the Kahun papyrus (1900 BC), etc[12] but the best known Egyptian pharmaceutical record is the “Ebers Papyrus” contain more than 800 formulae such as gargles, snuffs, poultices, infusions, pills and ointments, with beer, milk, wine and honey being commonly used as vehicles and 700 different drugs like acacia, castor oil and fennel etc with their uses along with apparent reference to some chemical such as iron, sodium chloride and sulphur. Chinese people have their own system since ancient time. Which consist manly drugs from plant origin used after small processing i.e. soaking in vinegar or wine. More than half the population regularly uses traditional remedies, with the highest prevalence of use in rural areas. About 5000 traditional remedies are available in China; they account for approximately one fifth of the entire Chinese pharmaceutical market.

2. PLANT PROFILE

This is a Jungle Flame (*Ixora coccinea*), also known as Jungle Geranium or Flame of the Woods. It is a dense, evergreen shrub that typically reaches 4–6 feet in height, though it can grow up to 12 feet. The plant produces small, tubular scarlet flowers in dense, rounded clusters. It is native to Southern India, Bangladesh, and Sri Lanka.

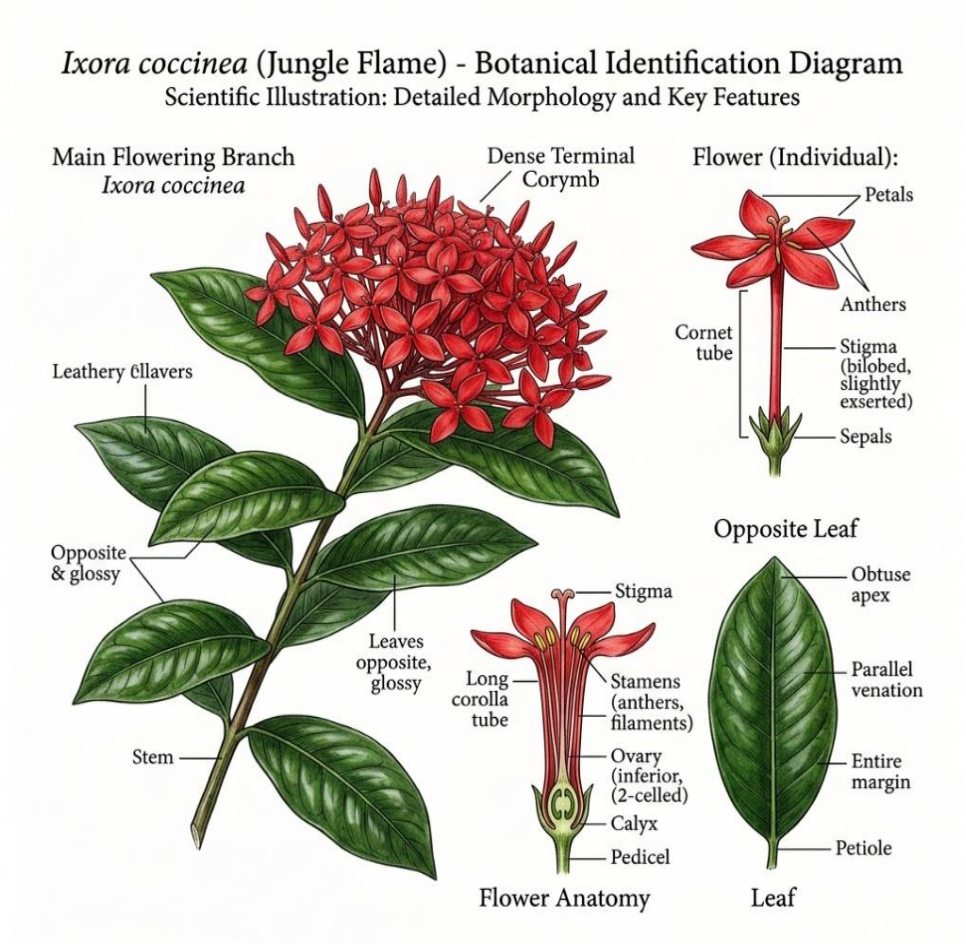


Fig: 1 Ixora coccinea.

- **Type:** Ever green perennial
- **Common name:** Jungle Flame
- **Family:** Rubiaceae
- **Kingdom:** Plantae
- **Clade:** Embryophytes
- **Clade:** Tracheophytes
- **Clade:** Spermatophytes
- **Clade:** Angiosperms
- **Clade:** Eudicots

- **Clade:** Asterids
- **Order:** Gentianales
- **Family:** Rubiaceae
- **Genus:** Ixora
- **Species:** I. coccinea

Chemical Constituents: Ixora coccinea (Jungle Flame) contains a wide range of bioactive phytochemicals that contribute to its medicinal and antioxidant properties. Phytochemical investigations have shown that the plant is rich in total phenolic compounds (approximately 3–8% dry weight) and total flavonoids (approximately 1–4% dry weight), depending on the plant part, extraction solvent, and geographical location. The flowers are particularly rich in anthocyanins (0.2–1.0%), which are responsible for their bright red colour and potent antioxidant activity. The leaves and roots also contain tannins (2–6%), saponins (1–3%), triterpenoids (0.5–2%), alkaloids (less than 1%), glycosides (0.5–2%), and phytosterols such as β-sitosterol and stigmasterol (0.1–0.5%). In addition, small amounts of carbohydrates, proteins, amino acids, vitamins, and minerals are present. The high concentration of phenolic compounds and flavonoids is mainly responsible for the plant's strong antioxidant activity by scavenging free radicals, reducing oxidative stress, and protecting cells from oxidative damage. It should be noted that the exact percentages vary among different plant parts, growing conditions, and extraction methods, but phenolics and flavonoids consistently represent the major antioxidant constituents of Ixora coccinea.

3. MATERIALS AND METHODS

3.1 PROCUREMENT AND AUTHENTICATION

The fresh plant materials of *Ixora coccinea* were collected from a suitable natural source and carefully selected to ensure that they were free from contamination and physical damage. The collected plant material was properly cleaned and processed for further study. The botanical identity of *Ixora coccinea* was confirmed and authenticated by National Siddha Institute, Arumpakkam, Chennai.

3.2 EXTRACTION OF PLANT MATERIALS

The collected plant materials were first cleaned thoroughly to remove dirt, dust, and other foreign materials and then properly dried under suitable conditions. The dried materials were subsequently pulverized into a coarse powder to increase the surface area for efficient extraction. The powdered plant material was subjected to solvent extraction using a Soxhlet

apparatus with a suitable solvent. After completion of the extraction process, the extracts were concentrated by removing the solvent under controlled conditions. Finally, the percentage yield of each extract was calculated based on the weight of the dried extract obtained in relation to the initial weight of the plant material used.

3.3 PHYTOCHEMICAL SCREENING

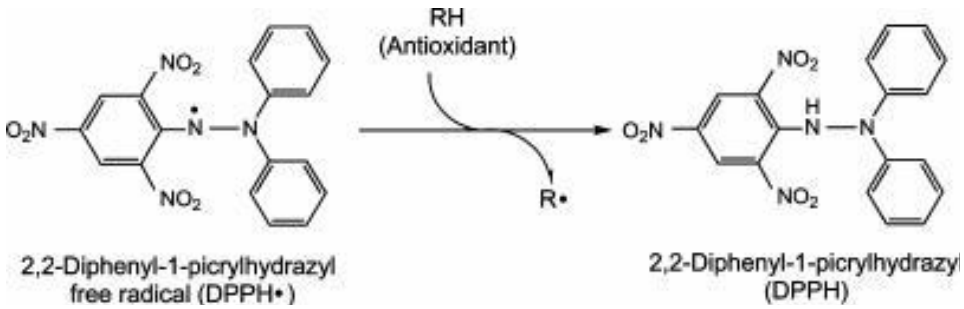
Phytochemical	Chemical Test	Positive Result
Alkaloids	Dragendorff's test	Orange or reddish-brown precipitate
	Mayer's test	Cream or white precipitate
Flavonoids	Shinoda test (Mg + HCl)	Pink or red coloration
	Alkaline reagent test	Intense yellow colour that disappears with acid
Tannins	Ferric chloride test	Blue-black or greenish-black colour
Phenolic compounds	Ferric chloride test	Deep blue, green or violet colour
Saponins	Foam test	Stable persistent froth for 10–15 minutes
Glycosides	Keller–Killiani test (for cardiac glycosides)	Brown ring at the interface with bluish-green upper layer
Terpenoids	Salkowski test	Reddish-brown colouration at the interface
Steroids	Liebermann–Burchard test	Green or bluish-green colour

Note: These tests are used for the preliminary identification of phytoconstituents and results may vary with solvent and concentration of the extract.

3.4 ANTI-OXIDANT ASSAY

Principle

The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. Figure 1, below, shows the mechanism by which DPPH accepts hydrogen from an antioxidant. DPPH is one of the few stable and commercially available organic nitrogen radicals (1). The antioxidant effect is proportional to the disappearance of DPPH in test samples. Monitoring DPPH with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm.



Material Required

0.1mM DPPH solution, Ascorbic acid, Methanol

0.1 mM DPPH Solution:

Dissolve 39 mg of DPPH in 100 ml of methanol and store at -20° C until needed.

Ascorbic acid (Standard)

1mg/ ml of Ascorbic acid

Procedure

1 Briefly, prepare 0.1 mM of DPPH solution in methanol and add 100 µl of this solution to 300 µl of the solution of Sample (MR) at different concentration (500, 250, 100, 50 and 10 µg/ml).

2 The mixtures have to be shaken vigorously and allowed to stand at room temperature for 30 minutes.

3 Then the absorbance has to be measured at 517 nm using a UV-VIS spectrophotometer. (Ascorbic acid can be used as the reference).

4 Lower absorbance values of reaction mixture indicate higher free radical scavenging activity.

5 The capability of scavenging the DPPH radical can be calculated by using the following formula.

6. DPPH scavenging effect (% inhibition) = [(absorbance of control- absorbance of reaction mixture)/absorbance of control] X 100

4. RESULTS AND DISCUSSION

4.1 EXTRACTION & YIELD

Table: 1 Extraction & Yield.

Parameter	Observation
Plant material	Selected plant material
Weight of powdered material	100 g
Extraction method	Soxhlet extraction
Solvent used	70% Ethanol
Duration of extraction	6–8 hours
Weight of concentrated extract	12.5 g
Percentage yield	12.5%
Physical appearance	Dark greenish-brown semisolid
Odour	Characteristic

4.2 PHYTOCHEMICAL SCREENING

Table: 2 Phytochemical Screening.

PHYTOCHEMICAL	CHEMICAL TEST	POSITIVE RESULT
Alkaloids	Dragendorff's test	Orange or reddish-brown precipitate
Alkaloids	Mayer's test	Cream or white precipitate
Flavonoids	Shinoda test (Mg + HCl)	Pink or red coloration
Flavonoids	Alkaline reagent test	Intense yellow color that disappears with acid
Tannins	Ferric chloride test	Blue-black or greenish-black color
Phenolic compounds	Ferric chloride test	Deep blue, green, or violet color
Saponins	Foam test	Stable persistent froth for 10–15 minutes
Glycosides	Keller–Killiani test (for cardiac glycosides)	Brown ring at the interface with bluish-green upper layer
Terpenoids	Salkowski test	Reddish-brown coloration at the interface
Steroids	Liebermann–Burchard test	Green or bluish-green color

4.3 ANTI-OXIDANT ASSAY

A. OD Value at 517 nm

Control Mean OD value: 1.285

Table: 3 Control Mean OD value: 1.285.

S. No	Tested sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)		
1.	Control	1.276	1.283	1.297
2.	500 µg/ml	0.201	0.203	0.207
3.	250 µg/ml	0.215	0.223	0.227
4.	100 µg/ml	0.632	0.641	0.648
5.	50 µg/ml	0.713	0.718	0.721
6.	10 µg/ml	0.791	0.795	0.801
7.	Ascorbic acid	0.104	0.107	0.112

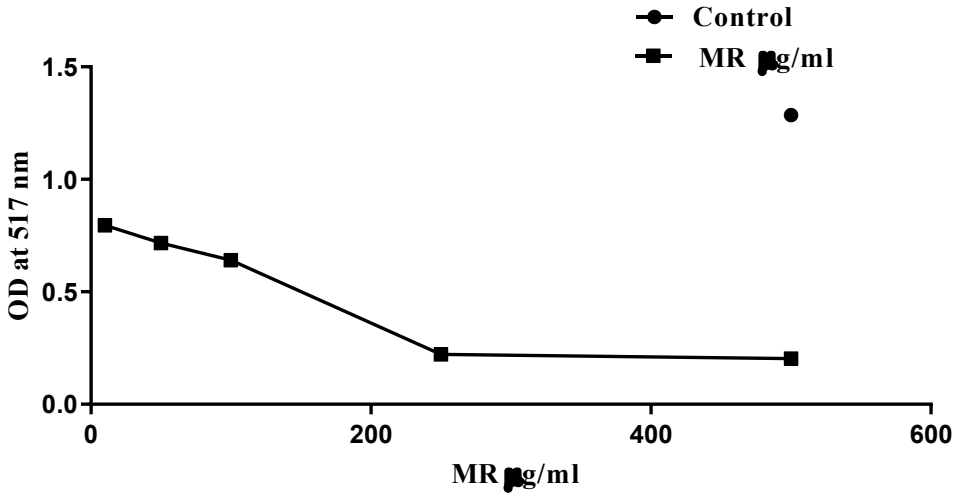


Fig: 3 Control Mean OD value: 1.285.

Table: 4 Percentage of Inhibition.

S. No	Tested sample concentration (µg/ml)	Percentage Of Inhibition (In Triplicates)			Mean value (%)
1.	Ascorbic acid	91.909	91.699	91.286	91.631
2.	500 µg/ml	84.336	84.206	83.895	84.146
3.	250 µg/ml	83.268	82.646	82.335	82.750
4.	100 µg/ml	50.817	50.117	49.572	50.169
5.	50 µg/ml	44.514	44.125	43.891	44.176
6.	10 µg/ml	38.444	38.132	37.665	38.080

B. Percentage of Inhibition

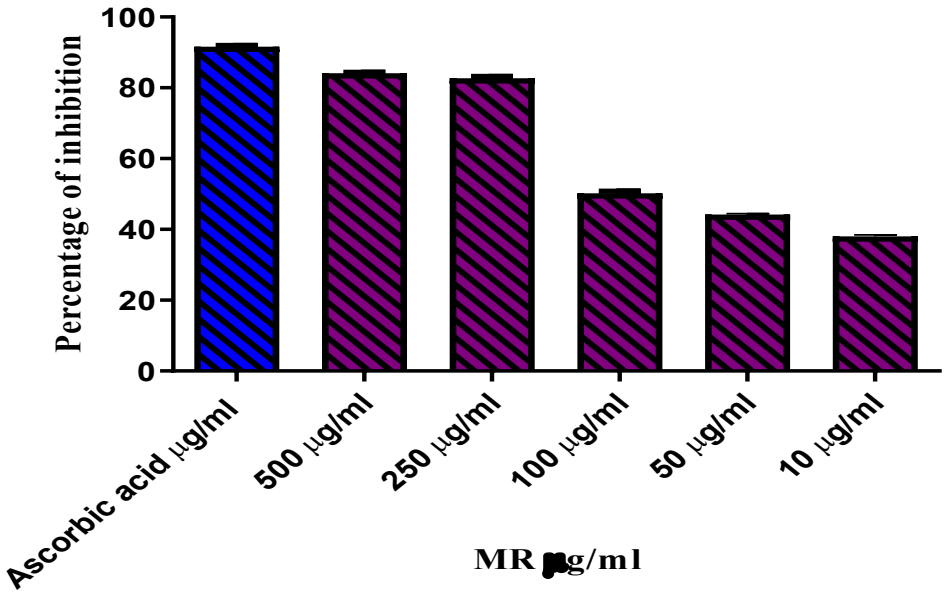


Fig: 4 Percentage of Inhibition.

C. IC50 Value of Tested Sample: 126.3 µg/ml

Table: 5 IC50 Value.

Log (inhibitor) vs. normalized response -- Variable slope	
Best-fit values	
LogIC50	**2.101**
HillSlope	**-3.942**
IC50	**126.3**
95% CI (profile likelihood)	
LogIC50	**2.059 to 2.144**
HillSlope	**-5.181 to -2.703**
IC50	**114.5 to 139.3**
Goodness of Fit	
Degrees of Freedom	**13**
R squared	**0.9853**
Sum of Squares	**401.1**
Sy.x	**5.555**
Number of points	
# of X values	**15**
# Y values analyzed	**15**

DPPH- MR

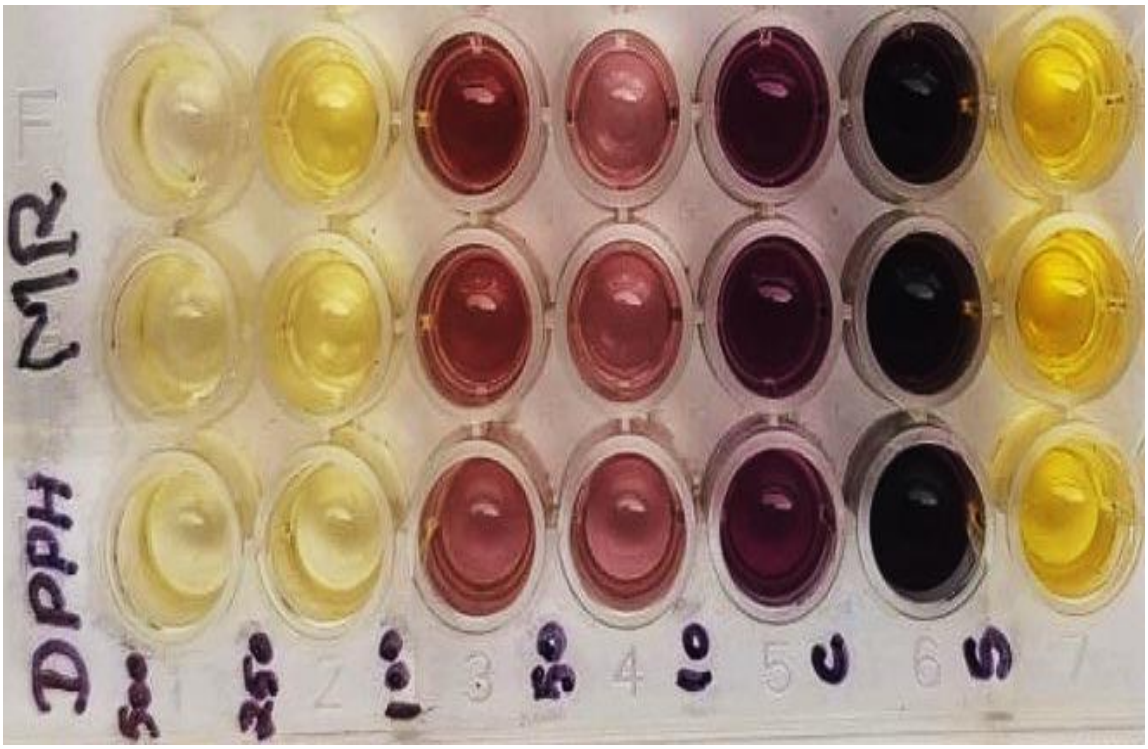


Fig: 5 Shows The Results of DPPH Assay.

DISCUSSION

The present investigation was carried out to evaluate the phytochemical constituents and antioxidant potential of *Ixora coccinea* extract. Proper procurement and authentication of medicinal plants are essential steps in pharmacognostic and phytochemical investigations because correct botanical identification ensures the reliability and reproducibility of the study. The plant material used in the present investigation was authenticated by the National Siddha Institute, Arumbakkam, Chennai, confirming its botanical identity as *Ixora coccinea*.

The plant material was subjected to cleaning, drying, pulverization, and Soxhlet extraction. Pulverization increases the surface area of the plant material and facilitates better contact between the powdered material and extraction solvent. In the present study, 100 g of powdered plant material was extracted using 70% ethanol for approximately 6–8 hours. A concentrated extract weighing 12.5 g was obtained, corresponding to a percentage yield of 12.5%. The obtained extract was dark greenish-brown in appearance and possessed a characteristic odour. The yield indicates that the extraction procedure was effective in recovering soluble constituents from the plant material.

Preliminary phytochemical screening revealed the presence of several important groups of secondary metabolites. Alkaloids were indicated by positive Dragendorff's and Mayer's tests,

while flavonoids were detected by Shinoda and alkaline reagent tests. Tannins and phenolic compounds showed positive reactions with ferric chloride. Saponins were detected by the formation of persistent foam, whereas glycosides, terpenoids, and steroids were indicated by their respective chemical tests. The presence of these phytoconstituents is important because many of these compound classes, particularly phenolics and flavonoids, are associated with antioxidant and free radical scavenging properties.

The antioxidant activity of the extract was evaluated using the DPPH radical scavenging assay. DPPH is a stable free radical that exhibits strong absorbance at 517 nm. In the presence of antioxidant compounds capable of donating hydrogen or electrons, the DPPH radical is reduced, resulting in a decrease in absorbance and a corresponding change in colour. Therefore, a reduction in absorbance indicates free radical scavenging activity.

The results demonstrated that the *Ixora coccinea* extract exhibited concentration-dependent antioxidant activity. At 10 µg/ml, the extract showed a mean inhibition of 38.08%. The inhibition increased to 44.18%, 50.17%, 82.75%, and 84.15% at concentrations of 50, 100, 250, and 500 µg/ml, respectively. This progressive increase indicates that higher concentrations of the extract were more effective in scavenging DPPH radicals. The standard antioxidant, ascorbic acid, produced 91.63% inhibition under the experimental conditions.

The IC_{50} value of the extract was found to be **126.3 µg/ml**, with a 95% confidence interval of approximately 114.5–139.3 µg/ml. The R^2 value of **0.9853** indicates a strong relationship between extract concentration and antioxidant response. The observed antioxidant activity may be related to the presence of flavonoids, phenolic compounds, tannins, and other reducing constituents identified during preliminary phytochemical screening. These compounds may donate hydrogen atoms or electrons to neutralize free radicals.

Overall, the findings demonstrate that the ethanolic extract of *Ixora coccinea* possesses appreciable DPPH radical scavenging activity. However, the DPPH assay represents an **in vitro antioxidant screening method**, and further investigations using additional antioxidant assays and appropriate chemical characterization are required to establish the antioxidant potential and identify the active constituents of the extract.

5. CONCLUSION

The present study demonstrated that the 70% ethanolic extract of ***Ixora coccinea*** contains several important classes of phytoconstituents and exhibits appreciable in vitro antioxidant activity. The extract showed a concentration-dependent increase in DPPH radical scavenging activity, with a maximum inhibition of 84.15% at 500 µg/ml and an IC_{50} value of 126.3

µg/ml. The strong concentration-response relationship, reflected by an R^2 value of 0.9853, supports the antioxidant potential of the extract. The observed activity may be associated with the presence of phenolic compounds, flavonoids, tannins, and other antioxidant phytoconstituents. Therefore, *Ixora coccinea* may serve as a promising natural source of antioxidant compounds. Further studies involving quantitative phytochemical estimation, chromatographic characterization, additional antioxidant assays, and in vivo evaluation are recommended to establish its therapeutic potential.

6. REFERENCES

1. Baliga MS, Kurian PJ. *Ixora coccinea* Linn.: traditional uses, phytochemistry and pharmacology. Chinese Journal of Integrative Medicine. 2012;18(1):72–79. doi:10.1007/s11655-011-0881-3.
2. Saha MR, Alam MA, Akter R, Jahangir R. In vitro free radical scavenging activity of *Ixora coccinea* L. Bangladesh Journal of Pharmacology. 2008;3(2):90–96. doi:10.3329/bjp.v3i2.838.
3. Idowu TO, Ogundaini AO, Salau AO, et al. Doubly linked, A-type proanthocyanidin trimer and other constituents of *Ixora coccinea* leaves and their antioxidant and antibacterial properties. Phytochemistry. 2010;71(17–18):2092–2098. doi:10.1016/j.phytochem.2010.08.018.
4. Annapurna J, Amarnath PVS, Amar Kumar D, Ramakrishna SV, Raghavan KV. Antimicrobial activity of *Ixora coccinea* leaves. Fitoterapia. 2003;74(3):291–293. doi:10.1016/S0367-326X(03)00037-6.
5. Ragasa CY, Tiu F, Rideout JA. New cycloartenol esters from *Ixora coccinea*. Natural Product Research. 2004;18(4):319–323. doi:10.1080/14786410310001630519.
6. Latha PG, Panikkar KR. Cytotoxic and antitumour principles from *Ixora coccinea* flowers. Cancer Letters. 1998;130(1–2):197–202. doi:10.1016/S0304-3835(98)00140-2.
7. Latha PG, Chandralekha CT, Vilasini G, Panikkar KR. Effects of the flower extract of *Ixora coccinea* Linn. on the meristematic cells of *Allium cepa*. Ancient Science of Life. 1998;17(4):262–267.
8. Ratnasooriya WD, Deraniyagala SA, Bathige SDNK, et al. Antinociceptive action of aqueous extract of the leaves of *Ixora coccinea*. Biologia Futura. 2005;56:21–34. doi:10.1556/ABiol.56.2005.1-2.3.
9. Gopalkrishnan B, Chiranjeev R. Pharmacognostical study of *Ixora coccinea* flower. Pharmacognosy Journal. 2018;10(5):1042–1046. doi:10.5530/pj.2018.5.176.

10. Elumalai A, Eswaraiah C, Venkatesh Y, Shiva Kumar B, Narendar C. Phytochemical and pharmacological profile of *Ixora coccinea* Linn. International Journal of Pharmacy and Life Sciences. 2012;3(3):1563–1567.
11. Dontha S, Kamurthy H, Mantripragada B. Phytochemical and pharmacological profile of Ixora: a review. International Journal of Pharmaceutical Sciences and Research. 2015;6(2):567–584. doi:10.13040/IJPSR.0975-8232.6(2).567-84.
12. Joshi H, Dutta S, Gururaj MP, Joshi AB, Chandrashaker KS, Dinesh Nayak M. A review on a potential herb: *Ixora coccinea* Linn. Research & Reviews: A Journal of Pharmacognosy. 2015;2(1):1–14.
13. Fasna Fasal K, Jimsha EV, Kunnool H, Krupa S. A review on *Ixora coccinea*: uses, properties and pharmacological studies. Journal of Pharmacognosy and Phytochemistry. 2024;13(5):298–301. doi:10.22271/phyto.2024.v13.i5d.15091.
14. Sivaraj C, Kumaraguru AJ, Saraswathi K, Arumugam P. Pharmacological activities of aqueous flower extract of *Ixora coccinea* L. Journal of Drug Delivery and Therapeutics. 2019;9(2-S):121–126. doi:10.22270/jddt.v9i2-s.2440.
15. Nwachukwu F, Muhammad I, Kulawe D, Yuguda UA. A study of the phytochemical constituents and the antioxidant effects of the leaf and flower of *Ixora coccinea* L. Jewel Journal of Scientific Research. 2019;4(1–2).
16. Idowu TO, Ogundaini AO, Salau AO, et al. Phytochemical constituents and antioxidant properties of *Ixora coccinea* leaves. Phytochemistry. 2010;71:2092–2098.
17. Latha PG, Panikkar KR. Antitumour activity of *Ixora coccinea* flower extract. Journal of Ethnopharmacology. 1999;66:1–6.
18. Nayak BS, Raju SS, Orette FA, Rao AV. Effects of *Ixora coccinea* flower extract on wound healing in experimental animals. Fitoterapia. 2003;74:291–295.
19. Ratnasooriya WD, Deraniyagala SA, De Silva W. Anti-inflammatory activity of *Ixora coccinea* leaf extract. Journal of Ethnopharmacology. 2005;97:375–379.
20. Nayak BS, Pinto Pereira LM. Catharanthus roseus flower extract and wound healing activity. BMC Complementary and Alternative Medicine. 2006;6:41.