ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

Ixora coccinea: Study of Phytochemical Parameters and Antioxidant Activity

Sneha G. Nair [1], Vikram R. Jadhav[2] & Satyam S. Bakare[3]

Assistant Professor, Department of Microbiology, K. K. Wagh Arts, Commerce, Science and Computer Science College, Kakasahebnagar, Nashik, India[1]

Assistant Professor, Department of Chemistry, K. K. Wagh Arts, Commerce, Science and Computer Science College, Kakasahebnagar, Nashik, India[2,3]

ABSTRACT: The species belonging to the genus *Ixora* are amongst the plants in Indian traditional Ayurveda system of medicine for a variety of ailments. The research work carried out extraction of *Ixora coccinea* plant, qualitative and quantitative analysis of *Ixora coccinea* plant extracts, antioxidant activity by DPPH method. Preliminary qualitative phytochemical screening gives a clue for the medicinal aptitude of the herb. In the conducted study bioactive compounds that impart biologically active nature to the plant were screened and results ensured the presence of phytochemical parameters phenols, alkaloids, flavonoids, terepnoids, coumarins, tannin, saponin, anthocynin, anthraquinone and amino acids.

KEYWORDS: *Ixora* coccinea, Phyto-chemical parameters, Antioxidant.

I. INTRODUCTION

Photo plate No 1. *Ixora coccinea* plant

Ixora is a genus of flowering plants in the Rubiaceae family. Red *Ixora* flowers are commonly used in Hindu worship as well as in Indian folk medicine. *Ixora coccinea* is a dense, multi-branched evergreen shrub, comely 4-6 ft in height,

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

but capable of reaching up to 12 ft as shown in photo plate no. 1. It has a rounded form, with a spread that may exceed its height. The glossy, leathery, oblong leaves are about 4 inch long, with entire margines, and are carried in opposite pairs or whorled on the stems. Small tabular, scarlet flowers in dense rounded clusters 2-5 inch across are produced almost all year long. It is used for diverse pharmacological properties including anti-inflammatory, antioxidant [2], and antimitotic activities [9].

Taxonomic classification of *Ixora coccinea*

- **Kingdom:** ***Plantae***
- **Subkingdom:** ***Viridae plantae***
- **Division:** ***Tracheophyta***
- **Class:** ***Magnoliopsida***
- **Super order:** ***Asteranae***
- **Order:** ***Gentianales***
- **Family:** ***Rubiaceae***
- **Genus:** ***Ixora***
- **Species:** ***coccinea***

Phytochemical Screening of *Ixora coccinea*:

The major phytoconstituents such as alkaloids, glycoside, flavonoids, tannins, saponins, steroids, terpenoids, etc are present in the *Ixora coccinea* [1]. 54 compounds have been identified in the oil of *Ixora coccinea* flower representing 99.97% of the total compounds. The oil is composed mainly of triterpenes 62.60%, monoterpenes 31.73%, sesquiterpenes 3.35% and an ester 2.29%. The anticancer activity of the leaves of *Ixora coccinea* was found to be principally due to the known alkaloid, camptothecin [10]. The presence of camptothecin was confirmed by RP-HPLC analysis [2, 3]. The average content of Camptothecin both in mature and young leaves was 2.8% and paves way for new findings. The chemical investigation of the roots which leads to the isolation of six phyto-constituents namely: 9, 12-octadecadienoic acid, di-n-octyl, phthalate, β- amyrin, kaempferol-7-oglucoside, kaempferitrin and quercetin.

II. MATERIALS AND METHODS

1. Extraction of *Ixora coccinea* plant:

1) ***Collection of the plant material****:* The leaves, stem and flower part of the *Ixora coccinea* was collected from HAL Ojhar T/S Nashik in Maharashtra State.

2) ***Preparation of the plant extract:*** Soxhlet method, Overnight extraction method, Fresh aqueous extraction method and Boiled extraction method.

2. Qualitative and Quantitative analysis.

Qualitative phytochemical screening:

The Screening of the soxhlet and overnight extracted plants were done according to the standard protocols

- ***Test for Flavonoid (Shinoda Test)***: A qualitative test was performed by adding mg-chips and few drops of concentration HCL to the extract. Appearance of reddish colour indicates the presence of flavonoid.
- ***Test for Coumarin (NaOH Test)***: To the 2 ml of plant extract 3 ml of 10% NaOH solution was added, formation of yellow colour indicates the presence of coumarin.
- ***Test for Anthocynin***: To the 2ml of plant extract, 2 ml of ammonia and few drops of 2N concentrated HCl was added. Appearance of bluish violet colour indicates the presence of anthocynin.
- ***Test for Terpenoid (Copper Acetate test)***: To the extract, 1 ml of copper acetate solution was added, formation of emrald green colour indicates the presence of terpenoid.

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

- ***Test for Alkaloids (Wagner's reagent test)***: 1 ml of wagner's reagent was added to the extract, formation of reddish brown precipitate indicates the presence of alkaloids.
- ***Test for Phenol (FeCl$_3$ Test and Lead acetate test)***: FeCl$_3$ Test- To the extract, 1ml of 10% FeCl$_3$ solution was added, formation of green precipitate indicates the presence of phenols.

Lead acetate test- 10% lead acetate solution was added on the plant extract, development of pale yellow colour indicates the presence of phenols.

- ***Test for Tannins ($K_2Cr_2O_7$ test)***: To the 0.5 ml of the extract, few drops of $K_2Cr_2O_7$ solution was added. Formation of red precipitate indicates the presence of tannin.
- ***Test for Saponin (Foam test)***: To the 2 ml of plant extract, 1 ml of distilled water was added and shaken vigorously for the formation of stable persistent froth, development of the froth indicates the presence of saponins.
- ***Test for Antraquinone (Borntrager's test)***: 1 ml of the extract was shaken well in 2 ml of chloroform. Equal volume of 100% ammonia solution was added. It was mixed well, the development of pink, violet or red colour in the ammonical layer indicates the presence of anthroquinones.
- ***Test for Amino acids (Ninhydrin test)***: To 1 ml of the extract, few drops of 55 ninhydrin solution were added. It was then boiled in water bath for 5 minutes. The appearance of bluish purple colour indicates the presence of amino acids.

Flavoniod is one of the most important biological compound of *Ixora coccinea* plant which have antimicrobial activity. Presence of flavonoid was confirmed by thin layer chromatography.

3. Quantitative analysis of Flavonoid by HPLC method.

Flavonoid is one of the most important biological compound of *Ixora coccinea* plant. During this study, quantitative analysis of the flavonoid was done by HPLC to quantify the concentration of flavonoid in the plant extract. Methanolic Leaf extract and flower extract (1:10) were used for this analysis. Detector used for this study was UV: 3000M, column Greece C-18 (4.6 ID 250 nm), particle size 5 micron, and pump used was P: 3000 M reciprocating (40MPa). Quercetin was used is as a standard for the confirmation of presence of flavonoid in the methanolic *Ixora coccinea* plant extracts. Peak area of the sample was calculated and flavonoid was quantified.

4. Antioxidant activity of *Ixora coccinea* plant extract by DPPH method.

On different concentration of the extracts (100 -500 micro g/ml), DPPH (Science lab) solution was added and then incubated for 30 minutes in a dark condition after incubation readings were taken from UV spectrophotometer at 517 nm.

	Conc. (µg/ml)	Stock (µl)	Solvent (µl)	DPPH (µl)	Incubation (Min)
	100	18	1982	1000	30
	200	36	1964	1000	30
	300	55	1945	1000	30
	400	73	1927	1000	30
	500	100	1900	1000	30
Blank		-	2000	1000	30

Table No. 1. Protocol for DPPH (Science lab) Scavenging antioxidant activity for methanolic leaf extract (Stock concentration of leaf extract is 22000 micro g ml).

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

Protocol for DPPH assay of leaf extract is shown in table no.1.

Concentration (µg/ml)	Stock (µl)	Solvent (µl)	DPPH (µl)	Incubation (Min)
100	42	1958	1000	30
200	84	1916	1000	30
300	126	1874	1000	30
400	168	1832	1000	30
500	211	1789	1000	30
Blank	-	2000	1000	30

Table No 2. Protocol for DPPH (Science lab) Scavenging antioxidant activity for methanolic stem extract (Stock concentration of stem extract is 9500 µg/ml).

Protocol for DPPH assay of stem extract is shown in table no. 2

Conc. (µg/ml)	Stock (µl)	Solvent (µl)	DPPH (µl)	Incubation (Min)
100	11.26	1988	1000	30
200	22.52	1978	1000	30
300	33.8	1966	1000	30
400	45.06	1955	1000	30
500	56.32	1944	1000	30
Blank	-	2000	1000	30

Table No. 3. Protocol for DPPH (Science lab) Scavenging antioxidant activity for methanolic flower extract (Stock concentration of flower extract is 35500 µg/ml.

Protocol for DPPH assay of flower extract is shown in table no. 3.

Formula: DPPH (Science lab) scavenging effect (% inhibition) = $\{A_0\text{-}A_1/A_0\}*100$

A_0 = Absorbance of control

A_1 = Absorbance of extract.

5. Extraction of *Ixora coccinea* plant:

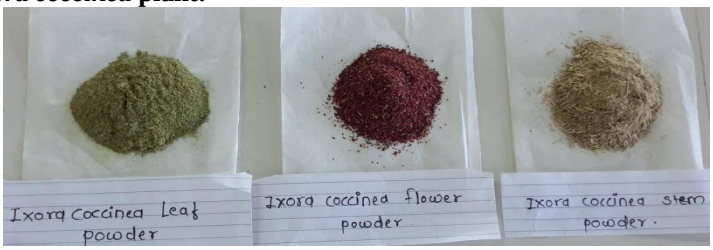


Photo plate No. 2: *Ixora coccinea* leaf, flower and stem powder.

Leaf, flower and stem were shade dried and converted into powder formed is shown in photo plate no. 2, Which was used for further analysis.

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

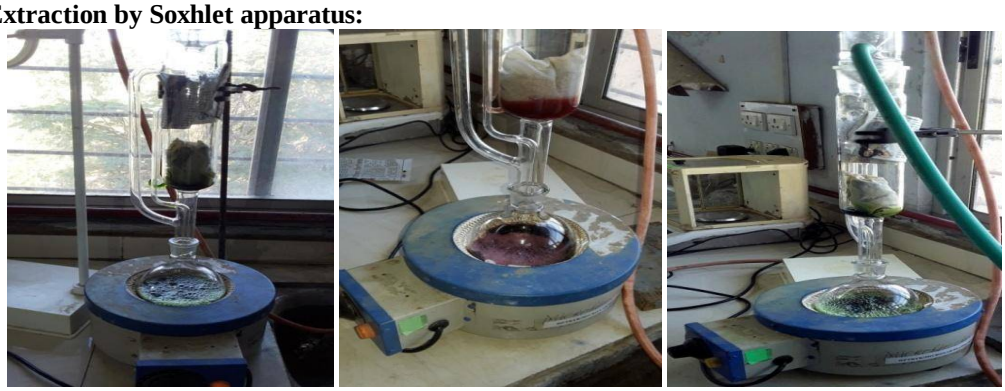
International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

6. Extraction by Soxhlet apparatus:

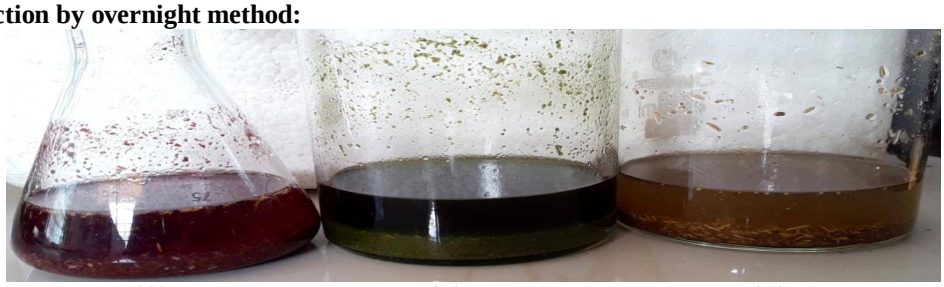


(A) (B) (C)

Photo plate No. 3: Extraction by soxhlet apparatus (A) Extraction of stem, (B) Extraction of flower, (C) Extraction of leaf

The Soxhlet apparatus is used for extraction of Stem, flower and leaf is shown in photo plate no. 3.

7. Extraction by overnight method:

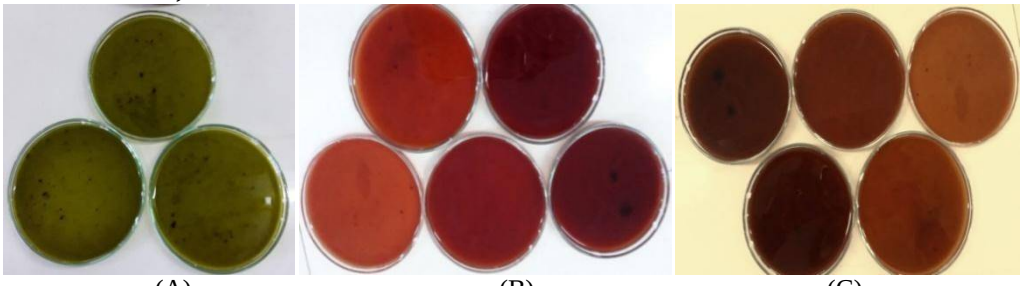


(A) (B) (C)

Photo plate No. 4: Extraction by overnight method **(**A) Flower extraction, (B) Leaf extraction, (C) Stem extraction.

Flower extraction, leaf extraction and stem extraction of *Ixora coccinea* by overnight is shown in photo plate no.4.

8. *Ixora coccinea* leaf, flower and stem extracts:



(A) (B) (C)

Photo plate No. 5: *Ixora coccinea* leaf, flower and stem extracts (A) Ethanolic leaf extract, (B) Ethanolic flower extract, (C) Ethanolic stem extract.

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

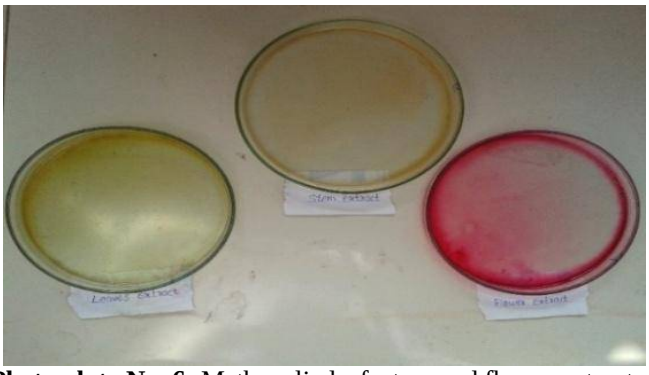


Photo plate No. 6: Methanolic leaf, stem and flower extract

By following above four extraction methods, *Ixora coccinea* plant parts such as leaf, stem and flower were extracted and used for further analysis.

III. EXPERIMENTAL RESULTS AND DISCUSSION

1. Qualitative Phytochemical Screening of *Ixora coccinea* plant extract:

Phytochemicals	Activity
Flavonoid	+++
Coumarin	+++
Anthocynin	+++
Terepnoid	+++
Alkaloid	+++
Phenol	+++
Tannin	+++
Saponin	++
Anthraquinone	++
Amino acid	++

Table No. 4. Results of Phytochemical screening +++: Highly positive, ++: Positive & +: Weak positive

Activity of phytochemical parameters of *Ixora coccinea* plant is shown in table no. 4**.**

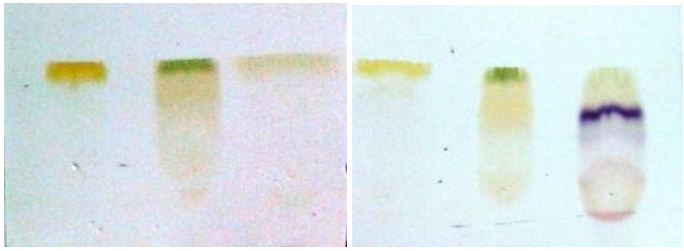


Photo plate No. 7. Spots developed after developing reagent, S – Standard (Quercetin), L- Leaf, s- Stem, F- Flower

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

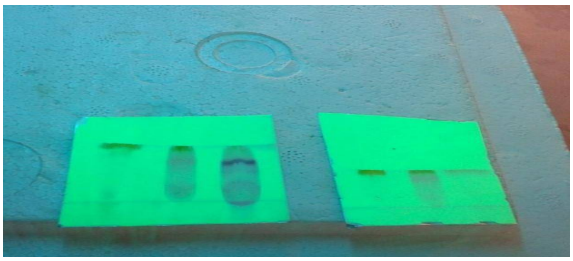
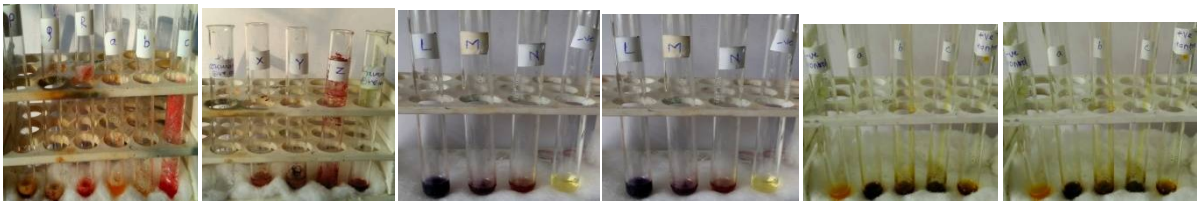


Photo plate No. 8. TLC of *Ixora coccinea* plat extracts.

TLC of flavonoid under UV light of Ixora coccinea plant extract of stem, flower and leaf is shown in photo plate no. 8.

2. Qualitative Analysis of Phytochemical Screening



Test for flavonoid

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

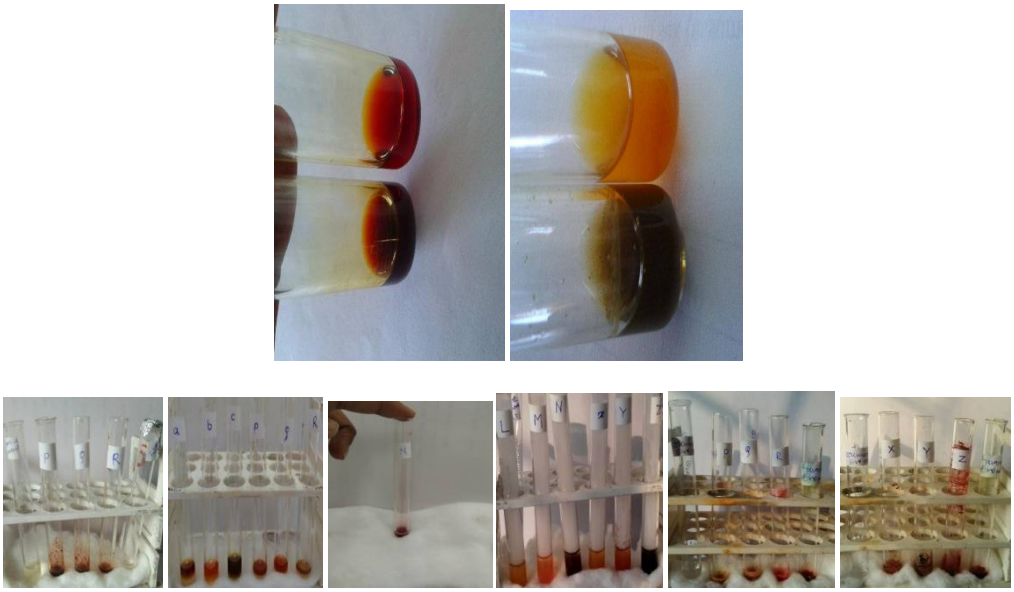


Photo plate No. 9. Qualitative analysis of *Ixora coccinea* plant extract

Qualitative analysis of phytochemical parameters such as flavonoid, amino acids, tannin, terepnoid, coumarin, alkaloid, phenol, anthraquinone, anthocynin, and saponin tests are shown in photo plate no. 9.

III. RESULTS OF PHYTOCHEMICAL SCREENING

Phytochemicals	A	B	C	P	Q	R	L	M	N	X	Y	Z
Flavonoid	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Coumarin	+++	+++	+++	++	+	++	++	+	++	+	+	++
Anthocynin	+	+	+++	+	+	+++	+	+	+++	+	+	+++
Terepnoid	++	+	+++	++	++	+++	++	+	+++	++	++	+++
Alkaloid	++	+	+++	+++	++	+++	++	++	+++	++	++	+++
Phenol	+++	+++	+++	+	++	+++	+++	+++	+++	+++	+++	+++
Tannin	++	+	+++	+++	++	+++	+++	+++	+++	+++	+++	+++
Saponin	+	+	++	+	+	+	++	+	++	++	+	++
Anthraquinone	++	+	+++	++	+	+++	+	++	+++	+	++	+++
Amino acid	+	++	++	++	++	++	+++	++	++	+	++	++

Table No. 5. Phytochemical Screening

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

A: - Overnight aqueous leaf lamina extract, B: - Overnight aqueous stem extract,
C: - Overnight aqueous flower extract, P: - Overnight ethanolic leaf lamina extract,
Q: - Overnight ethanolic stem extract, R: - Overnight ethanolic flower extract,
L: - Soxhlet aqueous leaf lamina extract, M: - Soxhlet aqueous stem extract,
N: - Soxhlet aqueous flower extract, X: - Soxhlet ethanolic leaf lamina extract,
Y:-Soxhlet ethanolic stem extract, Z:-Soxhlet ethanolic flower extract,
+++ :- Strong positive, ++ :- positive and + :- Weak positive

The soxhlet extracts both aqueous and ethanolic had better results as compared to over night (aqueous and ethanolic) extracts and hence supporting our hypothesis of soxhlet extraction method as the best extraction method which enhances the quality of extraction as shown in table no. 5. Preliminary phytochemical screening was performed for each extracts. It was noted that *Ixora coccinea* plant extracts contains flavonoid, coumarin, anthocynin, terpenoid, alkaloid, phenol, tannin, saponin, anthraquinone, amino acid as shown in photo plate no. 9 and table no. 5.

4. Detection of flavonoid by thin layer chromatography:

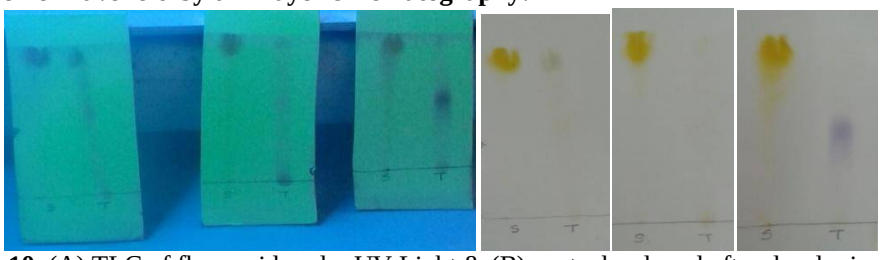


Photo plate No. 10. (A) TLC of flavonoid under UV Light & (B) spots developed after developing reagent, S –Standard (Quercetin), L-Leaf, s- Stem, F-Flower

Spots were observed on TLC sheet. During TLC, flavonoid (quercetin) was detected at Rf value 0.84. Presence of flavonoid was confirmed by comparing it with standard quercetin as shown in photo plate no. 10.

5. Preparative thin layer chromatography of flavonoid:

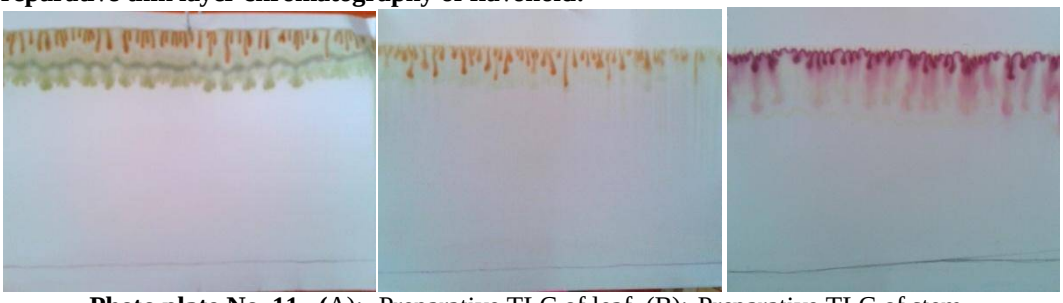


Photo plate No. 11. (A):- Preparative TLC of leaf, (B):-Preparative TLC of stem, (C):- Preparative TLC of flower

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

6. Purification of separated bands after preparative TLC:

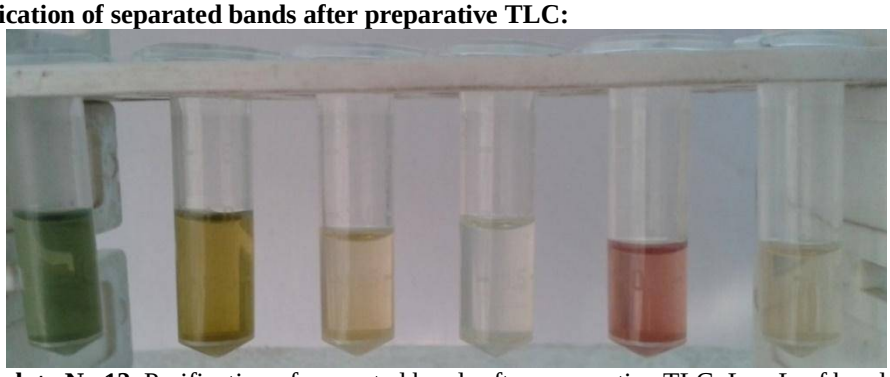


Photo plate No.12. Purification of separated bands after preparative TLC**,** La:- Leaf band a , Lb:- Leaf band b, Sa:- Stem band a, Sb:- Stem band b, Fa:-flower band a, Fb:- Flower band b

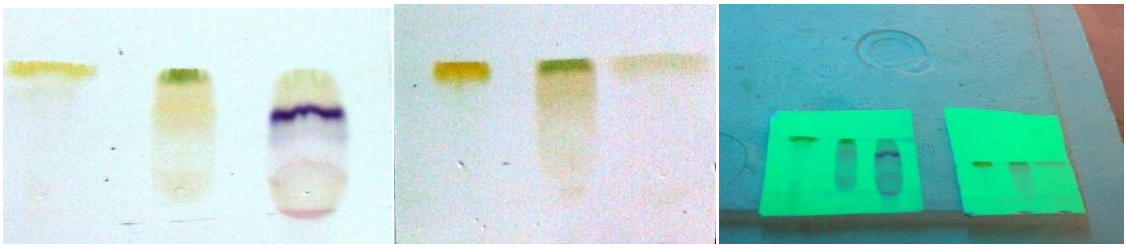


Photo Plate No.13. Comparison of crude extract and purified band.

7. **TLC of purified band:**

Photo plate No. 14. La: - Leaf band a , Lb:- Leaf band b, Sa:- Stem band a, Sb:- Stem band b, Fa:-flower band a, Fb: - Flower band b, S:-Standard, C: - Crude extract

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

8. Quantitative analysis of *Ixora coccinea* plant extract:
Total Phenol content by Folin Ciocalteau's method

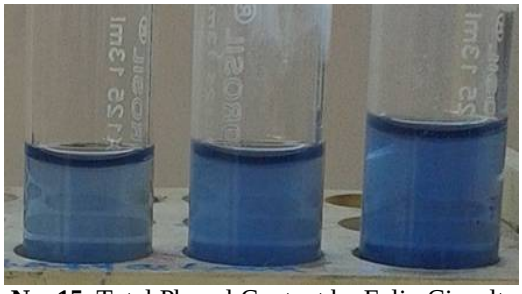


Photo Plate No. 15. Total Phenol Content by Folin Ciocalteau's method

Sample powder	Concentration(µg/mg)
Leaf	3.35
Stem	4.85
Flower	4.55

Table No. 5. Concentration of total phenol content in plant powder

Phenols play a vital role in the bioactivity of the extracts. The assay for the estimation of the total phenolic content using Folin Ciocalteau reagent showed that the stem powder has more total phenolic content than the leaf and flower powder as shown in photo plate no. 15.

9. **Total Flavonoid content by Aluminium Chloride Colorimetric Method:**

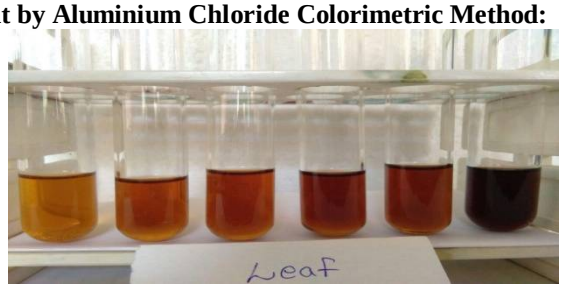


Photo Plate No. 16. Total Flavonoid content by Aluminium Chloride Colorimetric Method

Powder	Concentration(µg/mg)
leaf	3.35
Stem	4.85
Flower	4.55

Table No. 6. Concentration of total flavonoid content in plant powder

Flavonoids are important phytochemicals which are responsible for several activities. The assay for the estimation of the total flavonoid content using Aluminium Chloride as shown in photo plate no. 16 showed that the leaf powder has more total flavonoid content which was followed by flower and then stem powder.

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

10. HPLC Analysis of Flavonoid

Photo plate No. 17. HPLC Instrument

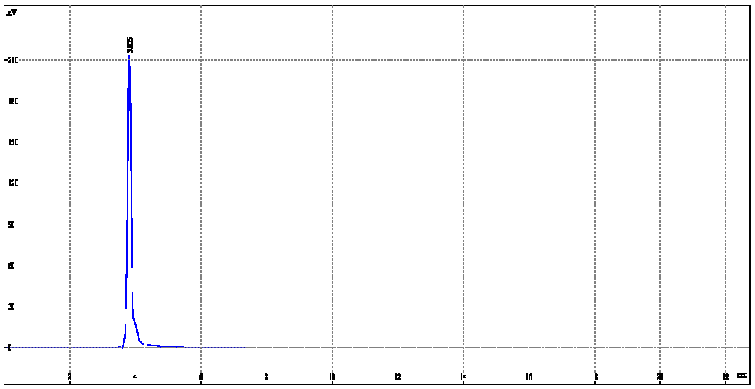


Figure No.1. Chromatogram of Standard (Quercetin)

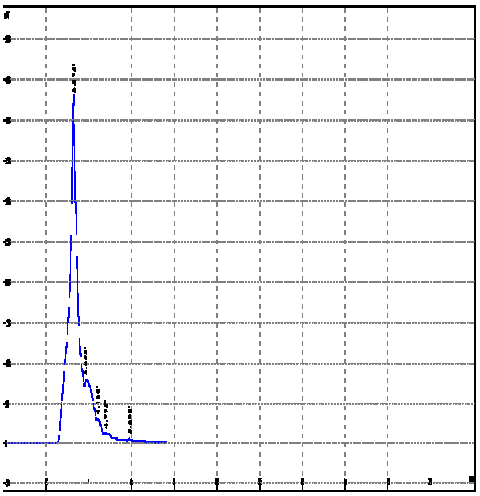


Figure No.2. Chromatogram of Leaf Extract

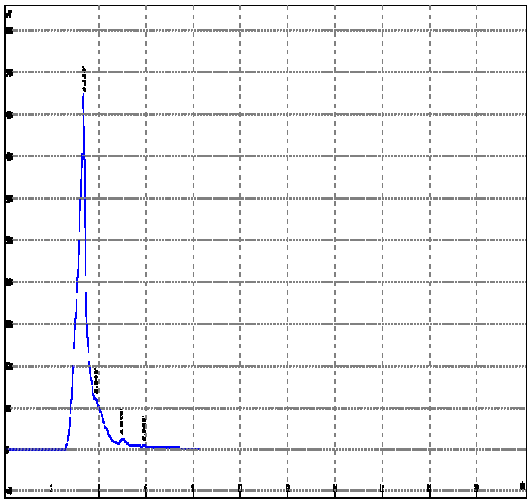


Figure No.3. Chromatogram of Flower extract

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

HPLC analysis of the leaf and flower showed a peak for flavonoid which is compared with the standard quercetin. The area under the peak was calculated. Concentration of leaf extract is 2.815 mg/ml and concentration of flower extract is 3.171 mg/ml as shown in figure no. 1, 2 and 3.

11. Antioxidant Activity of *Ixora coccinea* plant

a) Antioxidant assay by DPPH method (Leaf Extract)

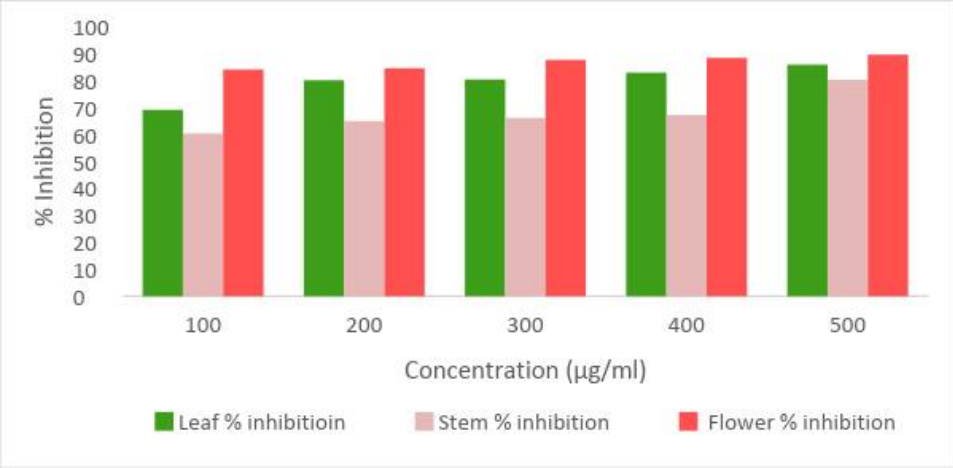


Figure No. 4. Antioxidant activity by DPPH method.

The results were indicated that all three extracts showed DPPH radical scavenging property. Flower extract had shown highest % inhibition as compared to leaf and stem extract as shown in figure No.4.

12. Statistical Analysis:

Statistical analysis of antioxidant activity of the plant extract was done.

Conc.	Leaf	Stem	Flower
100	69.24	60.46	84.21
200	80.17	65.00	84.53
300	80.42	66.17	87.74
400	83.00	67.30	88.45
500	86.00	80.37	89.70

Chi-square analysis:

- The chi-square statistic is 2.01. The P value is 9.49. This results is not significance at $P < 0.05$. Leaf shows less antioxidant activity.
- In stem, the Chi-square statistic is 3.26. The P-value is 9.49. This result is not significant at $P < 0.05$. Stem shows less antioxidant activity.
- In case of flower, the Chi-square statistic is 1391.23. The P-value is 9.49. This result is significant at $P > 0.05$. The flower shows highest antioxidant activity.

Pearson Correlation coefficient:

- The range of the correlation coefficient is from -1 to 1. Our result at 0.80 or 80%, it means Leaf and Stem have very strong positive correlation.
- The range of the correlation coefficient is from -1 to 1, our result is 0.81 or 81%, it means the stem and flower have a very strong positive correlation.

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

- The range of the correlation coefficient is from -1 to 1. Our result is 0.81 or 81%, it means the leaf and flower have a very strong positive correlation.

IV. CONCLUSION

This research is focused on qualitative quantitative analysis by phytochemical screening showed presence of different parameters like flavonoid, coumarin, tannin, saponin, anthocynin, anthraquinone, alkaloid, phenol, amino acid, terpenoids. During preparative TLC, flavonoid was detected as Rf value 0.84. HPLC analysis of flower and leaf showed presence of flavonoid where, concentration of flavonoid in flower and leaf was 3.171 mg/ml, 2.8155 mg/ml resp. During antioxidant analysis, strong radical scavenging activity was shown by flower which was 87.74% whereas leaf and stem showed comparatively weak activity which was 80.42% and 66.17% respectively.

REFERENCES

1. Baviskar J. W., Khandelwal S. R. (2015), Extraction, Detection and Identification of Flavonoids from Microalgae: An Emerging Secondary Metabolite, *International Journal of Current Microbiology and Applied Science*, PP.110-117.
2. Bose S., Maji S., Chakraborty P., (2013), Quercitrin from *Ixora coccinea* leaves and its Anti-oxidant activity, *International Journal of Pharmaceutical Sciences and Research*, Vol. No. 2, pp.72-74.
3. Dontha S., Kamurthy H. Mantripragada B.,(2015), Phytochemical and pharmacological profile of *Ixora*: a review, *International Journal of Pharmaceutical Sciences and Research*, Vol. 6, PP.567-584.
4. H. Sumathy, J. Sangeetha, K. Vijayalakshmi, (2011), Chromatographic fingerprint analysis of *Ixora coccinea* methanolic flower extract, *International Journal of Pharmaceutical Sciences and Drug Research* Vol.No. 3, pp. 327-330.
5. Kamtekar S., Keer V., Patil V.,(2014), Estimation of Phenolic content, Flavonoid content, Antioxidant and Alpha amylase Inhibitory Activity of Marketed Polyherbal Formulation, *Journal of Applied Pharmaceutical Science,* Vol. No. 4, pp. 61
6. Lachimanan, Latha Y., Darah I., Jain K., Sasidharan S., (2012), Pharmacological screening of methanolic extract of *Ixora* species, *Asian Pacific Journal of Tropical Biomedicine*,Vol No. 2, pp. 149-151.
7. Majid M., Khan M.R.,Shah N.A.,HaqUl N.,Farooq M.A.,Ullah S.,Sarif A.,Zahra Z.,Younis T. Sajid M.(2015),Studies on phytochemical,antioxidant,anti-inflammatory and analgesic activites of *Euphorbia dracuncuoides*, ***B****MC Complementary And Alternative Medicine*,pp.1-15.
8. Sabiha Sumbul, M. Aftab Ahmad, M. Asif, Mohd Akhtar,1 and Ibne Saud,(2012), Physicochemical and phytochemical standardization of berries of *Myrtus communis* Linn, *Journal of Pharmacology And Bioallied Science* ,Vol.No.4,pp.322
9. Muhammad Majid M., Khan M.R. Naseer Ali Shah2, Ihsan Ul Haq3, Muhammad Asad Farooq,Shafi Ullah, Anam Sharif, Zartash Zahra, Tahira Younis, Moniba Sajid, Studies on phytochemical, antioxidant, anti-inflammatory and analgesic activities of Euphorbia dracunculoides, *BMC Complementary and Alternative Medicine.*
10. Poornima Shyam P. Suresh P. K. (2013), Comparative analysis of three leaf extracts of *ixora coccinea* Linn. For their protective and anti-oxidant potentials and correlation with analytical data, *International Journal of Pharma and Bio Sciences*, Vol. No.4, pp. 937 – 949.