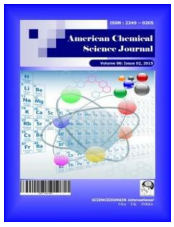

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Phytochemical Screening and Evaluation of *in-vitro* Anti-oxidant Activity of Extracts of *Ixora javanica* D. C Flowers

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Authors' contributions

This work was carried out in collaboration between all authors. Author HK designed the study, performed the statistical analysis. Author SD wrote the protocol and the first draft of the manuscript. Authors SD and HK managed the analyses of the study. Author BRM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Fruits and vegetables are the sources of nutraceutical compounds in human diet. However, flowers with their pigments rich in phenolics (phenolic acids, flavonoids and anthocyanins) are becoming an important source of several bioactive compounds.
Objective: To identify the compounds in *Ixora javanica* flowers and to test the *in vitro* antioxidant activity.
Materials and Methods: *Ixora javanica* flowers were collected, dried, powdered and extracted with solvents like petroleum ether (60-80°), ethyl acetate and ethanol, of increasing polarity by soxhilation. The phytochemical investigation and the determination of their use in traditional medicine, identified families of chemical compounds that guided us towards pharmacological studies like *in vitro* anti-oxidant activity. The crude extracts were tested for chemical identification of groups

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following the methods of Plummer, Trease and Evans etc. The *in vitro* antioxidant activity was tested throw DPPH free radical scavenging, superoxide radical scavenging and nitric oxide scavenging methods.

Results: The overall results of the phytochemical screening showed the presence of active constituents like flavonoids, polyphenols, anthocyanins, etc. The antioxidant potential of the ethanolic extract was high, whereas the ethyl acetate extract showed moderate activity and the petroleum ether extract showed poor activity.

Conclusion: The *in-vitro* anti-oxidant potential of the flower extracts is high. Further research is needed to be carried out to isolate and characterize the active constituents and to proceed for the *in-vivo* anti-oxidant activity also.

Keywords: Ixora javanica D.C flowers; flavonoids; antioxidant; DPPH; super oxide ion scavenging.

1. INTRODUCTION

The plants belonging to Rubiaceae family are generally a rich source of substances of phytochemical interest. Numbers of plants from this family are used in traditional system of medicine [1-2]. Ixora is a genus of flowering plants in the Rubiaceae family consisting of tropical evergreen trees and shrubs and holds around 500 species. Most of the species are grown as ornamental plants. Phytochemical studies of some other species like *I. coccinea* [3-5], *I. finalaysonia* [6-7]*, I. arborea* [8]*,* etc., indicated the presence of important phyto-chemicals [9-11] such as lupeol, ursolic acid, oleanolic acid, sitosterol, rutin, lecocyanadin, anthocyanins, proanthocyanidins, glycosides of kaempferol and quercetin. The investigations focused that the flower extracts were useful to prevent chronic degenerative diseases, especially those mediated by free radical damages. The great antioxidant activity indicates the potential of the extracts as a source of natural antioxidants or nutraceuticals with possible application to reduce oxidative stress with consequent health benefits. From the literature survey, it was revealed that no substantial work was carried out on *I. javanica* flowers both in the chemical investigation and pharmacological activities. Therefore, the objectives of the present study were to investigate the phytochemicals and screening of *in-vitro* antioxidant activity of flowers through the DPPH free radical scavenging, superoxide anion radical scavenging and nitric oxide scavenging methods. Hence an effort was made to investigate the different extracts and screening the anti-oxidant potential of *I. javanica* flowers.

2. MATERIALS AND METHODS

2.1 Plant Materials

Ixora javanica D.C flowers, were freshly collected from the local areas of Hyderabad (Osmania campus and Nursery), Telangana, India. The herbarium of *I. javanica* D.C was submitted to Dr. Bhadraiah, HOD, Department of Botany, Osmania University, Hyderabad and was authenticated by the Director of the same. The voucher specimen was preserved with reference number of the Authentication letter: MRCP/106/0599/ 2012 for future reference in Malla Reddy college of Pharmacy, Maisammaguda, Secunderabad, Telangana.

2.2 Chemicals and Equipment

2.2.1 Chemicals

1,1-Diphenyl-2-picryl hydrazyl (DPPH), Griess reagent and Dimethyl sulphoxide were obtained from MP Biomedicals Ltd., USA, Nitroblue tetrazolium (NBT), Riboflavin, Ascorbic acid and sodium nitroprusside were obtained from SD fine chemicals Ltd., India. Deoxy ribose was obtained from Merck India. Ethylene diamine tetra acetic acid (EDTA), ferrous sulphate, trichloro acetic acid, Hydrogen peroxide (H_2O_2), mannitol, potassium dihydrogen phosphate, potassium hydroxide, phenazine methosulfate were of analytical grade and obtained from Ranbaxy fine chemicals. Solvents like petroleum ether (60-80℃), ethyl acetate and ethanol (70%) and other chemicals were obtained from Finar chemicals.

2.2.2 Equipment

Soxhlet apparatus was used for extraction process and for determining absorbance UV-Visible spectrophotometer Shimadzu, UV-1601, Japan was used.

2.3 Extraction of Plant Materials

Five (5) kg of the plant flower material was thoroughly washed with clean water, dried at room temperature and powdered using a grinder.

About 0.2 kg of the powdered plant material was extracted with petroleum ether (60-80° boiling point), ethyl acetate and ethanol (70%) into 15 batches in Soxhlet extractor. After complete extraction, the different solvents were concentrated and finally dried under reduced pressure in a rotary flash evaporator. The residue (semisolid) was dried in a dessicator and kept in refrigerator for further study. After drying the respective extracts were weighed and percentage yield was recorded (Table 2).

2.4 Preliminary Phytochemical Investigation

Phytochemical analysis was performed on the basis of tests for coloring characteristics to highlight the major chemical groups. It focused on petroleum ether (60-80°), ethyl acetate and ethanol (70%) extracts. The general nature of the different phytochemicals like sterols, tannins, proteins, sugars, alkaloids, flavonoids, saponins, terpenoids, and cardiac glycosides was evaluated [12-14]. The chemical group tests were performed results are shown in Table 1.

2.4.1 Steroids

2.4.1.1 Libermann - Burchard's test

2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulfuric acid was added along the sides of the test tube. Formation of green colour indicated the presence of steroids**.**

2.4.1.2 Salkowski reaction

2 mg of dry extract was shaken with chloroform, and to the chloroform layer, sulphuric acid was added slowly by the sides of test tube. Formation of red colour indicated the presence of steroids.

2.4.2 Triterpenoids

2.4.2.1 Libermann - Burchard's test

2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a violet coloured ring indicated the presence of triterpenoids.

2.4.3 Flavonoids

2.4.3.1 Shinoda test

In a test tube containing 0.5 ml of the extract, 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicated the presence of flavonoids.

2.4.4 Saponins

In a test tube containing about 5 ml of extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicated the presence of saponins.

2.4.5 Carbohydrates

2.4.5.1 Anthrone test

To 2 ml of anthrone reagent solution, 0.5 ml of aqueous extract was added. Formation of green or blue colour indicated the presence of carbohydrates.

2.4.5.2 Benedict's test

To 0.5 ml of extract, 5 ml of Benedict's solution was added and boiled for 5 minutes. Formation of brick red coloured precipitate indicated the presence of carbohydrates.

2.4.5.3 Fehling's test

To 2 ml of extract, 1 ml mixture of equal parts of Fehling's solution A and B were added and boiled for few minutes. Formation of brick red coloured precipitate indicated the presence of reducing sugars.

2.4.5.4 Molisch's test

In a test tube containing 2 ml of aqueous extract, 2 drops of freshly prepared 20% alcoholic solution of α-naphthol was added. 2 ml of conc. sulphuric acid was added so as to form a layer below the mixture. Formation of red-violet ring, indicated the presence of carbohydrates, which disappeared on the addition of excess of alkali.

2.4.6 Alkaloids

2.4.6.1 Dragendroff's test

To 2 mg of the extract, 5 ml of distilled water was added, 2M Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendroff's reagent was added. Formation of orange red precipitate indicated the presence of alkaloids.

2.4.6.2 Hager's test

To 2 mg of the extract taken in a test tube, a few drops of Hager's reagent was added. Formation

of yellow precipitate confirmed the presence of alkaloids.

2.4.6.3 Wagner's test

2 mg of extract was acidified with 1.5% v/v of hydrochloric acid and a few drops of Wagner's reagent were added. Formation of yellow precipitate indicated the presence of alkaloids.

2.4.6.4 Mayer's test

To a few drops of Mayer's reagent, 2 mg of extract was added. Formation of pale yellow precipitate indicated the presence of alkaloids.

2.4.7 Proteins

2.4.7.1 Biuret's test

To 1 ml of hot extract, 5-8 drops of 10% w/v sodium hydroxide solution, followed by 1 or 2 drops of 3% w/v copper sulphate solution were added. Formation of violet red colour indicated the presence of proteins.

2.4.7.2 Millon's test

1 ml of aqueous extract was dissolved in 1 ml of distilled water and 5-6 drops of Millon's reagent were added. Formation of white precipitate, which turned to red on heating, indicated the presence of proteins.

2.4.8 Glycosides

2.4.8.1 Legal test

The extract was dissolved in pyridine, sodium nitroprusside solution was added to it and made alkaline. Pink colour was produced, which indicated presence of glycosides.

2.4.8.2 Keller Killani test

Sample was dissolved in acetic acid containing traces of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of liquid, a reddish brown colour was produced which gradually became blue.

2.4.9 Polyphenols

A drop of alcoholic solution of 2% ferric chloride was added to 2 mL of extracts. A blue-blackish coloration indicated a positive reaction.

2.4.10 Anthocyanins

5 ml of 10% H2SO4 was added to 5 ml of 5% extract, and then a base (5 drops of 25% NH_4OH) was added. The coloration is accentuated by acidification and then changed into blue-purplish in a basic environment, this indicates presence of anthocyanins.

Further the *in-vitro* anti-oxidant activity was performed by different models like DPPH radical scavenging, super oxide radical scavenging and nitric oxide methods.

2.5 Determination of *In vitro* Antioxidant Activity [15]

2.5.1 DPPH radical scavenging activity assay

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical-scavenging antioxidant) and is reduced to the DPPH and as consequence the absorbance's decreased from the DPPH. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured [16]. More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenyl picryl hydrazine; non radical) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present) [17].

DPPH (1, 1-Diphenyl–2-picrylhydrazyl) was dissolved in 3.3 ml methanol; it was protected from light by covering the test tubes with aluminum foil. 150 ml DPPH solution was added to 3 ml methanol and absorbance was taken immediately at 517 nm for control reading. 50 ml of different extracts as well as standard compound (Ascorbic acid) were taken and the volume was made uniformly to 150 ml using methanol. Absorbance was taken after 15 min. at 517 nm using methanol as blank on UV-visible spectrometer Shimadzu. The IC_{50} values for each extract as well as standard preparation were calculated. The DPPH free radical

scavenging activity was calculated using the following formula:

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations [18].

Fig. 1. *Ixora javanica* D.C flowers and leaves

2.5.2 Superoxide anion scavenging activity assay

The scavenging activity of various extracts of *I. javanica* flowers towards superoxide anion radicals was measured by colorimetric method [19]. Superoxide anion was generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments the superoxide anion was generated in 3 ml of Tris-HCl buffer (100 mM, pH 7.4) containing 0.75 mL of NBT (300 µM) solution, 0.75 ml of NADH (936 µM) solution and 0.3 ml of different concentrations of each extract. The reaction was initiated by adding 0.75 ml of PMS (120 µM) to the mixture. After 5 minutes of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The superoxide anion scavenging activity was calculated according to the following equation:

$$\% \text{ inhibition} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control (blank) and A_1 was the absorbance of the extract.

2.5.3 Nitric oxide scavenging activity assay [20]

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions [21], which can be determined by the use of the Griess Illosvoy reaction [22-23]. 2 ml of 10 mM sodium nitroprusside in 0.5 ml of phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25℃ for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml of sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthylene diamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radical scavenging activity was calculated according to the following equation:

$$\% \text{ inhibition} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance of the extract.

3. RESULTS AND DISCUSSION

3.1 Extraction of *Ixora javanica* D.C Flowers

After extraction of flowers with solvents of increasing polarity the colour and the percentage yield was reported (Table 1).

3.2 Phytochemical Analysis

The phytochemical investigation of the petroleum ether extract showed the presence of triterpenoids and sterols, whereas ethyl acetate extract the presence of flavonoids and polyphenols and finally the ethanolic extract indicated the presence of sterols, triterpenoids, flavonoids, carbohydrates, glycosides, polyphenols and anthocyanins (Table 2).

3.3 Anti-oxidant Results

3.3.1 DPPH radical scavenging activity

One of the quick methods to evaluate antioxidant activity is the scavenging activity on DPPH, a stable free radical to be widely used. In this method all the three extracts were evaluated for their free radical scavenging activity with ascorbic acid as standard compound. The IC_{50}

was calculated for each extract and summarized the results. The scavenging effect increased with the increasing concentrations of test compounds. The IC_{50} values of all the extracts were found to be comparatively lower than the IC_{50} of ascorbic acid.

3.3.2 Superoxide anion scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system. It was reported that superoxide anion scavenging activity of the leaf extracts from *I. coccinea* and *I. perviflora* assayed by the PMS-NADH system showed significant activity. The half inhibition concentration (IC_{50}) of petroleum ether, ethyl acetate and ethanolic extracts were calculated. The results indicated that all the extracts of *I. javanica* showed anti-oxidant potential.

Table 1. Percentage yield of different extracts of flowers of *Ixora javanica* D.C

S. no	Extracts	Nature of extract	Colour	Yield (%)
1.	Petroleum ether extract	Fine powder	Yellow orange	8.9
2.	Ethyl acetate extract	Sticky mass	Light yellow	6.7
3.	Ethanol (70%) extract	Sticky mass	Dark green	12.3

Table 2. Preliminary qualitative chemical investigation of different solvent extracts of *I. javanica* flowers

Chemical tests	Petroleum ether extract	Ethyl acetate extract	Ethanol (70 %) extract
1. Test for Sterols			
a. Salkowaski test	+	-	+
b. Liberman-Buchard test.	+	-	+
c. Sulphur test	+	-	+
2. Test for Triterpenoids			
a. Liberman-Buchard test.	+	-	+
3. Test for Flavonoids			
a. Shinoda test	-	+	+
4. Test for Saponins			
a. Foam test	-	-	+
5. Test for Carbohydrates			
a. Molish's test	-	-	+
b. Felhings test	-	-	+
6.Test for alkaloids			
a. Mayer's test	-	-	+
b. Wagner's test	-	-	+
c. Hager's test	-	-	+
d. Dragendrof's test	-	-	+
7. Test for Proteins			
a. Millons test	-	-	-
b. Biuret test	-	-	-
8. Test for glycosides			
a. Legal test	-	-	+
b. Kellar killani test	-	-	+
9. Test for Polyphenols	-	+	+
10. Test for Anthocyanins	-	-	+

Note: '+' Present, '_' Absent

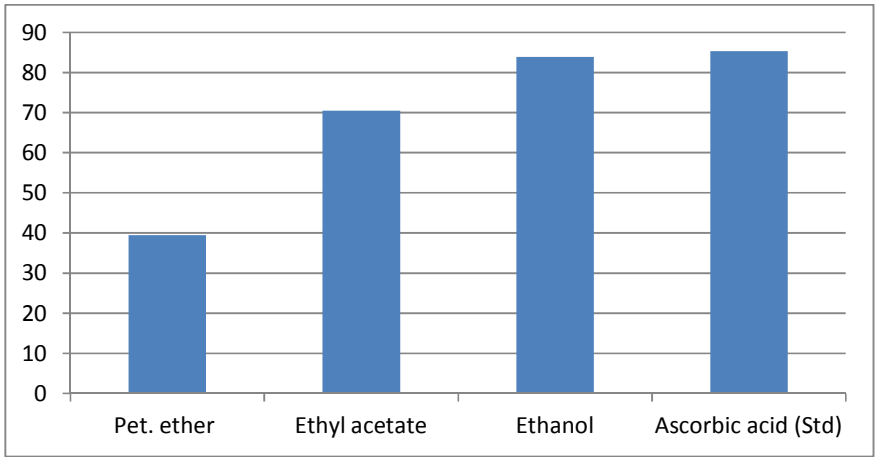

Fig. 2. DPPH radical scavenging activity

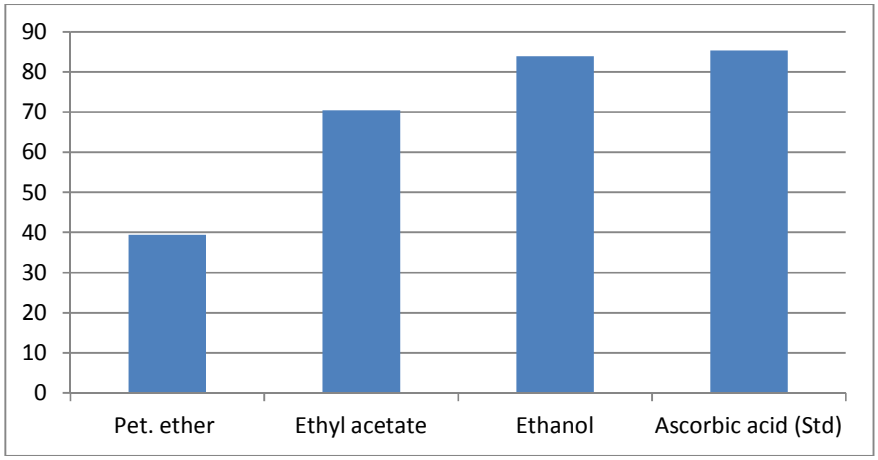

Fig. 3. Superoxide anion scavenging activity

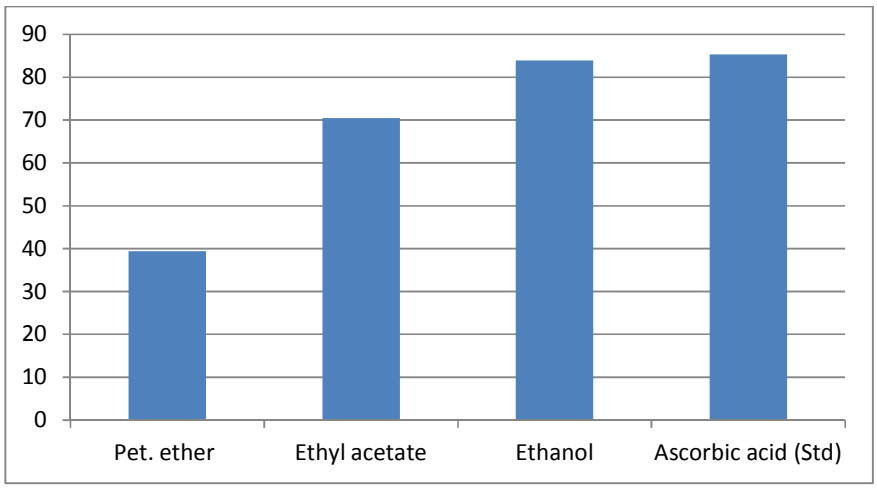

Fig. 4. Nitric oxide scavenging activity

Table 3. % inhibitions of various extracts by different methods

Extract	DPPH	Superoxide radical	Nitric acid
Petroleum ether	38.23	44.62	39.41
Ethyl acetate	70.02	71.43	70.42
Ethanol	71.41	78.23	83.90
Ascorbic acid (Std)	76.23	80.06	85.31

3.3.3 Nitric oxide scavenging activity

Nitric Oxide (NO) is a potent pleiotropic mediator of physiological process such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities. All the three extracts of *I. javanica* showed moderately inhibited nitric oxide scavenging activity in dose dependent manner.

The results showed that the anti-oxidant potential of the ethanolic extract was high due to the presence of flavonoids, sterols, polyphenols and anthocyanins, whereas the ethyl acetate extract showed moderate anti-oxidant activity due to the presence of flavonoids and polyphenols in all the methods compared with standard and finally the petroleum ether extract showed poor activity due to the presence of sterols and triterpenoids.

4. CONCLUSION

The present study suggested the presence of various phytoconstituents from different extracts of *Ixora javanica* D.C flowers. The results showed that the anti-oxidant potential of the flower extracts is high. It indicated that the selected medicinal plant is a better source of antioxidant which might be helpful to prevent the progress of oxidative stress. Further studies are to be focused on the isolation, characterization and purification of the active constituent and elucidating the exact mechanism of action.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kaisoon O, Siriamornpun S, Weerapreeyakul N, Meeso N. Phenolic compounds and antioxidant activities of edible flowers from Thailand. J. Funct. Foods. 2011;3:88–99.
2. Kirtikar KR, Basu BD, et al. Indian medicinal plants. 2nd ed. Oriental Enterprises: Dehradun, Uttaranchal, India; 2001.
3. Moni Rani Saha, Md. Ashraful Alam, Raushanara Akter, Rumana Jahangir. *In-vitro* free radical scavenging activity of *Ixora coccinea* L. Bangladesh J Pharmacol. 2009;3:90-96.
4. Sankhadip Bose, Sushomasri Maji, Pranabesh Chakraborty. Quercitrin from *Ixora coccinea* leaves and its anti-oxidant Activity. Journal of Pharma Sci Tech. 2013; 2(2):72-74.
5. Sankhadip Bose, Sushomasri Maji, Pranabesh Chakraborty. Comparative study of *in vitro* and *in vivo* antioxidant property of different Ixora species. Journal of Advanced Pharmacy Education & Research. 2011;2:90-103.
6. Faten Darwish MM, Zedan Ibraheim Z. Phytochemical Study of *Ixora finlaysoniana* Wall. Ex. G. Don growing in Egypt. Bull. Pharm. Sci. 2003;26(1):91-96.
7. Metcalfe CR, Chalk L. Anatomy of dicotyledones. The Clarendon Press. Oxford; 1970.
8. Aktar F, Kaisar A, Hamidul Kabir ANM, Hasan CM, Rashid MA. Phytochemical and biological investigations of *Ixora arborea* Roxb. Dhaka Univ J Pharm Sci. 2009;8:161–166.
9. Baliga MS, Kurian PJ. *Ixora coccinea* Linn: Traditional uses, phytochemistry and pharmacology. Chin J Integr Med. 2012; 18(1):72-79.
10. Gloria UO, Gibson UN. Chemical composition of essential oil of *Ixora coccinea* flower from Port harcourt, Nigeria. International Journal of Academic Research. 2011;3(2):381.
11. Ambreen I, Versiani MA, Shumaila S, Salman KA, Syed TA, Shaheen F. Ixorene, a new dammarane triterpene from the leaves of *Ixora coccinea* Linn. Rec. Nat. Prod. 2013;7(4):302-306.
12. Plummer DT. An introduction to practical biochemistry. 3rd ed. Tata Mc Graw-Hill Education; 1988.

13. Trease GE, Evans WC. Pharmacognosy. 15th ed. Saunders publishers: London; 2002.
14. Sofowora EA. Screening plants for bioactive agents. In: Medicinal plants and traditional medicinal in Africa. 2nd ed. John Wiley and Sons. London; 1993.
15. Patel Rajesh M, Patel Natvar J. *In vitro* antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. Journal of Advanced Pharmacy Education & Research. 2011;1:52-68.
16. Mohammad I, Zafar I, Javid H, Hidayat H, Manzoor A, Asma E, et al. Chemical constituents and antioxidant activity of *Geranium wallichianum*. Rec. Nat. Prod. 2009;3(4):193-197.
17. Bondet V, Brand Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. Food Science and Technology. 1997;30:609-615.
18. Iranshahi M, Askari M, Sahebkar A, Hadjipavlou LD. Evaluation of antioxidant, anti-inflammatory and lipoxygenase inhibitory activities of the prenylated coumarin umbelliprenin. DARU. 2009; 17(2):99-103.
19. Liu F, Ooi VEC, Chang ST. Free radical scavenging activity of mushroom polysaccharide extracts. Life Sci. 1997;60: 763-771.
20. Bose S, Bose A, Maji S, Chakraborty P. *In vitro* antioxidant property of leaf extract of *Ixora coccinea* L. International Journal of Biomedical and Pharmaceutical Sciences. 2008;2(2):84-87.
21. Govindarajan R, Rastogi S, Vijayakumar M. Studies on antioxidant activities of *Desmodium gangeticum.* Bio Pharm. Bull. 2003;26:1424-1427.
22. Garrat DC. The qualitative analysis of drugs. Chapman and Hall: Japan. 1964;3.
23. Bhatt LR, Baek SH. Antioxidant capacity of crude extract and fractions from *Woodfordia fruticosa* flower. Oriental Pharmacy and Experimental Medicine. 2007;7(2):162-170.

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