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# Antioxidant property of polysaccharides isolated from *Ixora coccinea* leaves

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**Abstract**
Three different fractions of polysaccharides (PS1, PS2, PS3) were isolated from *Ixora coccinea* leaves to evaluate their antioxidant activity. The percentage of sugar in the extracted samples was checked by phenol- sulphuric acid method. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) assays and total antioxidant assay were done to evaluate its antioxidant property. The PS1, PS2, PS3 showed a % inhibition of DPPH radical at 43.21%, 31.11% and 24.23%. Total antioxidant activity was found to be higher in PS1. Both PS2 and PS3 showed almost equal antioxidant activity. The results of the present study indicates that polysaccharides from *Ixora coccinea* helps to overcome the untoward effects of oxidative stress, thus, contributing to the development of new drugs.

**Keywords:** *Ixora coccinea*, DPPH assay, total antioxidant assay

## 1. Introduction
Plant polysaccharides have traditionally been used as folk remedy for various diseases due to their multiple biological properties including anti-inflammation, anti-hepathitis, anti-ulcer etc. They have attracted researchers by virtue of their renewable character, biodegradation, relatively low cost and possibility of conversion into various derivatives due to their reactivity with many organic molecules [1]. Among the macromolecules, polysaccharides offer the highest capacity for carrying biological information because they have a great potential for structural variability [2]. Although research on polysaccharides, has been limited, due to the various problems in isolation and purification procedures. In this study the polysaccharide fractions have been isolated and estimated.
*Ixora coccinea* (Rubiaceae) is globally distributed across India, Nepal, Bhutan, Myanmar, South East Asia, and China. It is a perennial, deciduous shrub attaining 2.5 m in height. The plant is used in Ayurveda and other folk medicines for the treatment of different diseases and disorders such as polydipsia, diarrhoea, diabetes, dysentery, fever and as acts as blood purifier. Applying the concept of ancient wisdom and modern science, many lead compounds can be identified which can be developed into drugs with least side effects. Isolation and purification process are found to be the two limitations factors in the polysaccharide research and evaluated their antioxidant activity [3].

## 2. Materials and methods
### 2.1 Isolation of polysaccharide fractions
*Ixora coccinea* leaves were procured from the Medicinal Plant Garden of Gupta College of Technological Sciences, Asansol West Bengal in the month of June and authenticated by Shibpur Botanical Garden, Shibpur, Kolkata. It was with water, rinsed with distilled water and blotted gently between the folds of filter paper. 60 g of leaves were taken and ground into a fine paste using mortar and pestle. For extraction, cold distilled water was added to the leaf paste and centrifuged. Supernatant was taken and stored at 4 °C. Hot distilled water was added to the debris obtained and agitated for 2 h in water bath set at 70-80 °C. It was then centrifuged and supernatant was collected. To another 30 g of leave paste, ethanol was added and kept in a shaker at 120 rpm for overnight. After the complete evaporation of ethanol, 1 mol/l of NaOH was added and agitated at 100 °C for 4 h. All the three types of solutions were dialysed separately against double distilled water using 12-14,000 MW membrane. It was then subjected to ethanol precipitation. The precipitate was dissolved in hot water. The process was repeated 3 times. Polysaccharide precipitate soluble in cold water (PC), hot water (PH) and hot NaOH (PA) was collected by centrifugation at 12,000 rpm at 4 °C, redissolved in distilled water and lyophilized [4-5].

### 2.2 Estimation of polysaccharide content

The sugar content in the different extracted fractions was estimated using the phenol-sulphuric acid method [6]. About 10 mg of sample was dissolved in 100 ml of distilled water. From this 1ml was used for sugar analysis. To estimate the polysaccharide content in sample, 1 ml of 5% phenol was added to the 1 ml of sample, followed by 5 ml of concentrated sulphuric acid. The absorbance was measured after 10 min at 488 nm against blank. The experiment was carried out in triplicates. Glucose was used as the standard.

### 2.3 Total antioxidant assay (TAA)

The total antioxidant assay is based on the reduction of molybdenum VI to molybdenum V to form a green phosphate complex [7-11]. Briefly 0.3 ml of different extracts ranging from 0.2-1 mg/ml concentrations were mixed with 3 ml of reagent solution (0.6 mol/l sulfuric acid, 0.028 mol/l sodium phosphate and 0.004 mol/l ammonium molybdate). Reaction mixture was incubated at 95 °C for 90 min in water bath. As the temperature drops down to normal, absorbance of each extract solution was marked at 695nm.

TAA is expressed as the number of equivalents of ascorbic acid.

### 2.4 DPPH radical scavenging assay

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) is a powerful free radical used to evaluate the electron donating capacity of antioxidants. DPPH is a stable free radical useful in the study of natural antioxidants [7-11]. The reaction mixture contained 2.8 ml of 100 µM DPPH dissolved in methanol and different concentrations ranging from 2 mg/ml of polysaccharide fractions in 0.2 ml DMSO. This mixture was incubated at room temperature for 30 minutes. After shaking the mixture, absorbance was measured at 517 nm. The percentage of DPPH scavenging activity was calculated as follows:

% DPPH scavenging activity = $[(A_{control} - A_{test})/ A_{control}] \times 100$

$A_{control}$ and $A_{test}$ represent absorbance of control and test respectively.

### 2.5 Statistical analysis

Data were expressed as the means ± standard deviations (SD) of the triplicate values.

## 3. Results and Discussion

### 3.1 Polysaccharide isolation

Three crude fractions of polysaccharides PS1, PS2 and PS3 were obtained with a yield of 1.95%, 1.82% and 1.81% respectively. All the three fractions were of neutral pH and soluble in water. PC was light brown in colour and the other two were light yellow in colour.

### 3.2 Estimation of sugar content

The sugar content in different samples was determined by the Phenol-Sulphuric acid method. The calibration curve for different concentrations of glucose is represented in figure 1. Using the proposed method, the calibration curve was found to be linear in the range of 0.1-0.7 mg/ml. The % Relative Standard Deviations (% RSD) lies between 0.30- 0.95 indicating that the method is precise. The sugar content of PS1, PS2, PS3 was calculated using regression equation obtained from the calibration curve.

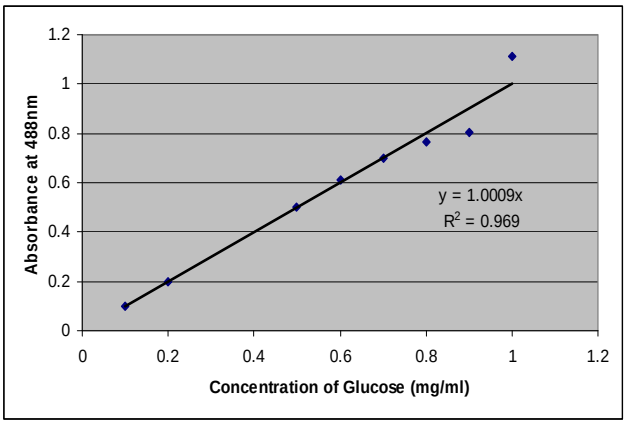


**Fig 1:** Calibration curve of different concentrations of glucose estimated by phenol- sulphuric acid method

**Table 1:** The absorbance shown by different crude polysaccharide fractions

| Sl no | Absorbance of PS1 | Absorbance of PS2 | Absorbance of PS3 |
|---|---|---|---|
| 1 | 1.134 | 0.106 | 1.016 |
| 2 | 1.133 | 0.105 | 1.013 |
| 3 | 1.123 | 0.104 | 1.010 |
| Mean | 1.13±0.006083 | 0.105±0.001 | 1.013±0.003 |
| Total Sugar Content | 1.13 mg/ml | 0.105 mg/ml | 1.012 mg/ml |
| % RSD | 0.54 | 0.95 | 0.30 |

From this, the total sugar content in PS1, PS2, PS3 was found to be 1.13 mg/ml, 0.105 mg/ml and 1.012 mg/ml respectively.

### 3.3 Total Antioxidant Assay

The total antioxidant assay is based on the reduction of molybdenum VI to molybdenum V to form a green phosphate complex. Fractions ranging from 0.5-2 mg/ml were tested for their antioxidant activity. The absorbance shown by the three fractions at different concentrations is represented in figure 3. All the three showed a dose- dependent increase in the antioxidant activity.

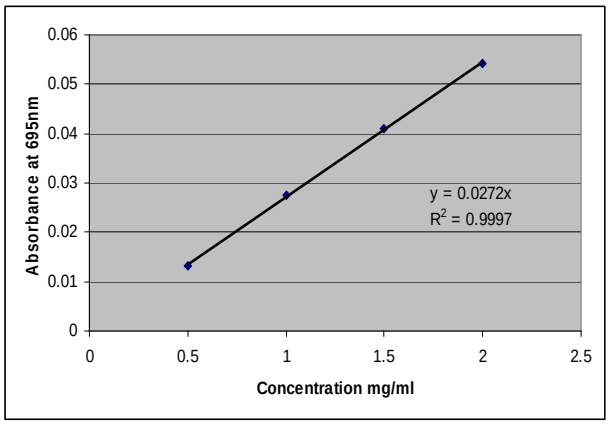


**Fig 2:** Calibration curve of different concentrations of total antioxidant assay

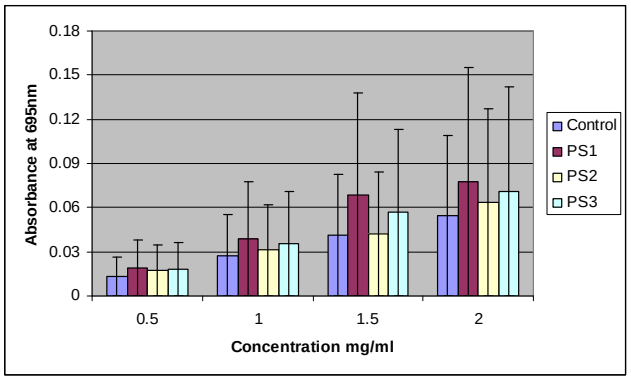


**Fig 3:** The absorbance shown by different crude polysaccharide fractions and the control in the total antioxidant assay

From the above results, it can be inferred that all the three samples exhibit antioxidant property. But the highest activity was shown by PS1 as compared to PS2 and PS3.

### 3.4 DPPH Assay

DPPH is a free radical which is used to evaluate the electron donating capacity of antioxidants. The extracts were able to reduce the stable pink coloured free radical DPPH to yellow colored diphenyl picryl hydrazine. Ascorbic acid was used as standard. PS1 was found to have the highest value of 43.21% DPPH scavenging activity at a concentration of 2 mg/ml where as PS2 and PS3 were found to be 31.11% and 24.23% DPPH scavenging activity at a concentration of 2 mg/ml respectively. In the DPPH assay also, PS1 was shown to have the highest activity when compared to PS2 and PS3.

## 4. Discussion

Plant polysaccharides with various biological properties especially their low toxicity and structure flexibility contribute a lot to modern medicine. Due to the arduous isolation and purification procedures, research on polysaccharides is comparatively less compared to other secondary metabolites. In this study, we have tried to isolate and evaluate the antioxidant properties of polysaccharides isolated from *Ixora coccinea* leaves.

The polysaccharides isolated were water soluble, neutral in pH and had a good yield. The total sugar content in PS1, PS2 and PS3 was found to be 1.13 mg/ml, 0.105 mg/ml and 1.012 mg/ml respectively. Phenol-sulphuric acid technique is a simple, precise and rapid spectrophotometric technique for the determination of total polysaccharides. Polysaccharides play a role in disease therapy by activating immune cells and the complement system; regulating the cytokines expression; promoting the production of antibodies; inhibiting tumor cell proliferation and inducing tumor cell apoptosis; inhibiting virus entering cells and replication; increasing activity of antioxidant enzyme; scavenging free radicals; and inhibiting lipid peroxidation [18]. All the three polysaccharides isolated from *Ixora coccinea* leaves were found to be non-toxic to normal cells.

Oxidative stress, induced by the oxygen radicals is believed to be a primary factor in the development of several degenerative changes in cells and tissues which ultimately lead to several degenerative disorders like arthritis, atheroscelorosis, cancer, neurodegeneration etc [20]. Body defences are not completely capable of protecting from the negative effects of the oxygen radicals. Compounds with antioxidant properties are promising in curing such diseases [21]. The total antioxidant and DPPH assay was done to evaluate the antioxidant capacity of the polysaccharides. In the total antioxidant assay, PS1 was found to have the highest activity as compared to PS2 and PS3. Increasing concentrations, lead to increasing activity. PS1 showed an antioxidant activity near to ascorbic acid used as standard. DPPH assay was also done to evaluate the scavenging capacity of the compounds. 1 mg/ml of PS1 shows a DPPH scavenging activity equivalent to 22.1 µg/ml of ascorbic acid. It indicates that it can act as an efficient hydrogen- donor like ascorbic acid. Antioxidants can be developed from this polysaccharide fractions for the treatment of various disorders associated with free radicals.

## 5. Conclusion

The present study investigates the antioxidant properties of the three fractions of polysaccharides isolated from *Ixora coccinea* leaves. Polysaccharides can be used as a potent drug in the treatment of many diseases due to their non-toxic and bio-degradable properties. The polysaccharides isolated from *Ixora coccinea* are water soluble and of good yield. Further studies are required to purify the active fractions and understand the detailed mechanism involved in their antioxidant mechanism. All the three fractions isolated have shown a good antioxidant capacity and has the ability to scavenge DPPH.

## 6. Acknowledgement

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