INVITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF ETHANOLIC LEAF EXTRACT OF IXORA COCCINEA

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ABSTRACT
The present study deals with the antioxidant potential of Ixoracoccinea leaves collected from Ibrahimpatnam of Krishna dist belonging to the family Rubiaceae. In this study we tried to explore the antioxidant activity by using DPPH, reducing power, and nitric oxide assay methods. The results are compared with standard Gallic acid. The results showed the appreciable and concentration dependent antioxidant activity.

Keywords: Ixoracoccinea, reducing power, nitric oxide scavenging assay, antioxidant activity.

INTRODUCTION
Free radicals and active oxygen species plays an important role in initiation and evaluation of numerous diseases2. Antioxidants are the first line of defense against free radical damage and are critical for maintaining optimum health. Antioxidants are emerging as prophylactic and therapeutic agents for various diseases like cancer [2, 3], diabetes, cardiovascular disorders, brain dysfunction, inflammation and other degenerative diseases2. Antioxidants delays the ageing and formation of white hairs in early age.

Ixoracoccinea is the evergreen perennial shrub through south East Asia belonging to family Rubiaceae. The genus ixora contains more than 400 species. Ixoracoccinea is commonly known as jungle of geranium and flame of the woods or vetchi in ayurveda4. Ixora coccinea have different colours of flowers like red, white, yellow, orange, and pink1. Leaves contain the chemical constituents5, 6 like A-type trimeric proanthocyanidin, epicatechin, procyanidine A2, cinnamatanninB-1, flavonols, kaemferol, quercetin, phenolic acids, ferulic acids, mixture of hydrocarbons, sesquiterpenes, steroids and alcohols, mixture of lupeol. Leaves have show antimicrobial7, antinociceptive8, antinflammatory9, hypolipidaemic and hypoglycaemic6, antimicrobial10, anti-ulcer5, chemoprotective activities4. Flowers contain quercetin, cyanidin, flavonoids, ash 6.4%, tannins, lupeol, fatty-acids, beta-sitosterols, cycloartenol-esters, triterpenes, monoterpenes, sesquiterpenes11. Flowers used as hepatoprotective, antidiarrheal, cytotoxic, antimicrobial, wound healing12 and anti-inflammatory effect. Roots are having aromatic acria oil, tannins, and fatty acids. Roots having anthelmintic13 and antibacterial activities. The present investigation was carried out to evaluate the antioxidant property of ethanol leaf extract of Ixoracoccinea.
MATERIALS AND METHODS

Plant material
The leaves of *Ixora coccinea* were collected from the surrounding areas of Vijayawada thermal power station [V.T.P.S] located at Ibrahimpatnam of Krishna district. The leaves were collected and shade dried and then powdered to get a coarse powder.

Preparation of extract
The dried powder materials was macerated by using 90% ethanol for five days and percolated for 50°C. The percolated mixture was filtered and filtrate was collected and solvent was evaporated. The residue was kept in desiccator.

Chemicals and equipment
Gallic acid [gifted sample], Dpph [purchased from research lab fine chemical industries, Mumbai], phosphate buffer [0.2M, PH 6.6], potassium ferricyanide, trichloroacetic acid, ferrichloride, methanol, sodium nitroprusside, sulphanilamide, H₃PO₄, naphthyl ethylene diaminedihydrochloride, distilled water, UV-Visible double beam spectrophotometer [elicosl 210], centrifuge machine, PH meter, shimadzu electronic balance.

Antioxidant assays
The following assays were performed for evaluation of antioxidant efficacy of the ethanolic leaf extract.

DPPH radical scavenging assay
DPPH [2, 2-diphenyl picrylhydrazyl] is commercially available commonly used stable free radical, which is purple in colour. Antioxidant molecules when incubated, reacts with dpph and converts to di-phenyl hydrazine which is yellow in colour. The degree of discoloration of purple to yellow was the measure of scavenging potential of plant extract which was measured at 520nm. Different concentrations of ethanolic leaf extract [100µg/ml, 200µg/ml, and 500µg/ml] is prepared. Gallic acid was taken as reference standard [1µg/ml, 2.5µg/ml, and 5µg/ml] was prepared 0.1 mM solution of dpph in methanol was prepared. 1ml of 0.1 mM dpph solution is mixed with 1ml of all concentrations of both plant extract and standard separately. These 0.1mM dpph solution and gallic acid in methanol mixture is taken as control [blank]. These mixtures are kept in dark for about 30minutes and optical density was measured at 520nm. The dpph radical scavenging activity of ethanolic leaf extract was calculated as the percentage inhibition.

\[ \% \text{ inhibition of Dpph radical} = \frac{A_0 - A_t}{A_0} \times 100 \]

Where A₀ is absorbance of blank, Aₜ is absorbance of test. Lower absorbance of the reaction mixture indicate higher free radical scavenging activity. The IC₅₀ and compared with standard. The IC₅₀ value was defined as concentration [1g/ml] of plant extract that scavenges the dpph radicals by 50%.

Reducing power
1 ml of various concentrations of ethanolic leaf extract solution was mixed with phosphate buffer [2.5ml, 0.2M, PH 6.6] and potassium ferricyanide [2.5ml, 1%]. After the mixture was incubate at 50°C for 20 min, trichloro acetic acid [2.5ml, 10%] was added and the mixture was centrifuged at 3000rpm for 10 min. The upper layer of solution [2.5ml] was mixed with distilled water [2.5ml] and ferric chloride [0.5ml, 0.1%] and then absorbance was measured at 700nm against a blank. Increased absorbance of reaction mixture indicated increased reducing power. Gallic acid was used for comparison as reference standard. All tests were performed in triplicate and mean values were contexed.

Nitric oxide scavenging assay
Nitric oxide has a pivotal role in the regulation of diverse physiological and pathophysiological process. Nitric oxide was generated by Greiss reaction[1% sulphanilamide, 0.1% naphthyl ethylene diamine dihydrochloride, 2% H₃PO₄] sodium nitroprusside in aqueous solution at physiological PH spontaneously generates
nitric oxide which interacts with oxygen to produce nitric ions that can be estimated by using Griess reagent scavengers of nitric oxide compete with oxygen leading to reduce production of nitric oxide. Sodium nitroprusside [5mM] in phosphate buffer saline was mixed with 3.0ml of different concentrations of ethanolic leaf extract [100µg/ml, 200µg/ml and500µg/ml] and incubated at 25ºc for 180min. the samples were added to Griess reagent. The absorbance of chromaphore formed during the diazotization of nitrite with sulphanilamide was read at 546nm and referred to absorbance of standard solutions of Gallic acid treated in the same way with Griess reagent as a positive control. The percentage of inhibition was measured similar to that of DPPH assay.

Table 1: DPPH antioxidant assay of *Ixoracoccinea* ethanolic leaf extract

<table>
<thead>
<tr>
<th>Tested material</th>
<th>Concentration (µg/ml)</th>
<th>% inhibition</th>
<th>IC₅₀ value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic leaf extract of <em>Ixoracoccinea</em></td>
<td>100</td>
<td>68 ± 0.005</td>
<td>25.73</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>76 ± 0.017</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>96 ± 0.0173</td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1</td>
<td>59.8 ± 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>68.16 ± 0.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>80.03 ± 0.024</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean ± SEM; n=3

Table 2: Nitric oxide assay of *Ixoracoccinea* ethanolic leaf extract

<table>
<thead>
<tr>
<th>Tested material</th>
<th>Concentration (µg/ml)</th>
<th>% inhibition</th>
<th>IC₅₀ value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic leaf extract of <em>Ixoracoccinea</em></td>
<td>100</td>
<td>83 ± 0.02</td>
<td>2.07</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>88 ± 0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>99 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1</td>
<td>50.53 ± 0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>69.84 ± 0.028</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>76.76 ± 0.0057</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean ± SEM; n=3

Table 3: Reducing power assay of *Ixoracoccinea* ethanolic leaf extract

<table>
<thead>
<tr>
<th>Tested material</th>
<th>Concentration (µg/ml)</th>
<th>Absorption ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic leaf extract of <em>Ixoracoccinea</em></td>
<td>100</td>
<td>0.1103 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.1741 ± 0.025</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.3808 ± 0.0038</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1</td>
<td>0.0439 ± 0.0015</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>0.0589 ± 0.0032</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0.0826 ± 0.005</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM; n=3
Fig. 1: DPPH antioxidant activity of Gallic acid (standard)

Fig. 2: DPPH antioxidant activity of *Ixoracoccinea* ethanolic leaf extract
Fig. 3: Nitric oxide antioxidant activity of *Ixoracoccinea* ethanolic leaf extract

![Graph showing nitric oxide antioxidant activity of *Ixoracoccinea* ethanolic leaf extract]

\[ y = 49.56x^{1.144} \]
\[ R^2 = 0.987 \]

Fig. 4: Nitric oxide antioxidant activity of Gallic acid (standard)

![Graph showing nitric oxide antioxidant activity of Gallic acid (standard)]

\[ y = 51.77x^{0.264} \]
\[ R^2 = 0.949 \]
RESULTS AND DISCUSSION
DPPH radical scavenging assay
The reduction capability of Dpph radicals was determined by the decrease in its absorbance at 520nm, which is induced by antioxidants. Table-1 shows the percentage of Dpph radical scavenged by gallic acid and ethanolic leaf extract at various concentrations [1g/ml]. Fig 1 and 2 illustrates a decrease in concentration of Dpph radical due to the scavenging ability of standard Gallic acid as reference compound and soluble constituents in the ethanolic extract of leaves of Ixoracoccinea presented the highest activity at all concentrations. The IC<sub>50</sub> values were found to be 25.73µg/ml and 0.39µg/ml for ethanolic leaf
extract of *Ixoracoccinea* and Gallic acid respectively.

**Nitric oxide scavenging activity**
Ethanolic leaf extract exhibited potential inhibiting activity against nitric oxide generation. Table-2 nitric oxide is a potent pleiotropic mediator of physiological process. The antioxidant activity of ethanolic leaf extract of *ixoracoccinea* showed 99% inhibition of nitric oxide generation at 500µg/ml which is compared with standard Gallic acid 5µg/ml concentration shown in fig 3 and 4. The IC$_{50}$ values were found to be 2.07µg/ml and 0.876µg/ml for the ethanolic leaf extraction of *Ixoracoccinea* and Gallic acid. The antioxidant activity increases with the increase in the concentration of extract.

**Reducing power**
Reducing power assay is used for the measurement of antioxidant activity in the extract. The reducing agents in the extract reacts with the potassium ferricyanide [Fe$^{3+}$] to form potassium ferrocyanide [Fe$^{2+}$], which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700nm. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Table-3 shows the reducing power of ethanolic leaf extract of *ixoracoccinea* from figure 5 and 6 it was found that the absorbance of the extract and Gallic acid increases with increase in concentrations. Reducing power capabilities of extract was found to be closer to Gallic acid.

**CONCLUSION**
The detected extensive antioxidant study on *ixoracoccinea* leaf extract revealed that it processes significant antioxidant activity. The presence of several phytoconstituents may be responsible for this activity. So these leaves of *ixoracoccinea* may be used as rational medicine and further studies can be carried out to explore the chemical constituents responsible for pharmacological studies.

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