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Research Article

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 Rubiacea. 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 andevaluation of numerous diseases[2]. 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, cardiovasculardisorders, braindysfunction, inflammation and other degenerative diseases[2].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 ixoracontains more than 400 species. *Ixoracoccinea* is commonly known as jungle of geranium and flame of the woods or vetchi in ayurveda[4].*Ixora coccinea* have different colours of flowers like red, white, yellow, orange,and pink[1]. Leaves contain the chemical constituents [5, 6] like A-type trimericproanthocyanidin, epicatechin, procyanidineA_2, cinnamatanninB-1,flavonols,kaemferol,quercetin,phenolic acids,ferulicacids, mixtureof hydrocarbons, sesquiterpens, steroids and alcohols, mixture of lupeol. Leaves have showantimicrobial[7],antinociceptive[8],antiinflammatory[9], hypolipidaemic and hypoglycaemic[6], antimicrobial10, anti-ulcer[5], chemoprotective activities[4]. Flowers contain quercetin, cyanidin, flavonoids,ash 6.4%, tannins, lupeol, fatty-acids, beta-sitosterols, cycloartenol-esters, triterpenes, monoterpenes, sesquiterpenes[11]. Flowers used as hepatoprotective, antidiarrheal, cytotoxic, antimicrobial, wound healing[12]and anti-inflammatory effect. Roots are having aromatic acria oil, tannins, and fatty acids. Roots having anthelmintic[13] 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*Ixoracoccinea* 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 powered 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^{0}c. The percolated mixture was filtered and filtrate was collected and solvent was evaporated. The residue was kept indescicator.

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, trichloroaceticacid,ferricchloride,methanol,sodiumnitroprusside,sulphanilamide,H_3PO_4, 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[2,3]

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 in to di-phenyl hydrazine which is yellow in colour. The degree of discoloration of purple to yellow was the measure ofscavenging 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 mMdpph 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.

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

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

Reducing power[2,3,15]

1 ml of various concentrations of ethanolic leaf extract solution was mixed with phosphate buffer [2.5ml, 0.2M, PH6.6] and potassium ferricyanide [2.5ml, 1%]. After the mixture was incubate at 50^{0}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 forcomparison as reference standard. All tests were performed in triplicate and mean values were contexed.

Nitric oxide scavenging assay[10,16]

Nitric oxide has a pivotal role in the regulation of diverse physiological and phathophysiological process. Nitric oxide was generated by Greiss reaction[1% sulphanilamide,0.1% naphthyl ethylene diamine dihydrochloride,2% H_3PO_4] 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/mi, 200µg/ml and500µg/ml] and incubated at 25°c 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

Tested material	Concentration (µg/ml)	% inhibition	IC_{50} value (µg/ml)
Ethanolic leaf extract of ixoracoccinea	100 200 500	68 ± 0.005 76 ± 0.017 96 ± 0.0173	25.73
Gallic acid	1 2.5 5.0	59.8 ± 0.05 68.16 ± 0.008 80.03 ± 0.024	0.391

Values are the mean ± SEM; n=3

Table2: Nitric oxide assay of *Ixoracoccinea*ethanolic leaf extract

Tested material	Concentration (µg/ml)	% inhibition	IC_{50} value (µg/ml)
Ethanolic leaf extract of ixoracoccinea	100 200 500	83 ± 0.02 88 ± 0.01 99 ± 0.005	2.07
Gallic acid	1 2.5 5.0	50.53 ± 0.02 69.84 ± 0.028 76.76 ± 0.0057	0.876

Values are the mean ± SEM; n=3

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

Tested material	Concentration (µg/ml)	Absorption ± SEM
Ethanolic leaf extract of ixoracoccinea	100 200 500	0.1103 ± 0.001 0.1741 ± 0.025 0.3808 ± 0.0038
Gallic acid	1 2.5 5.0	0.0439 ± 0.0015 0.0589 ± 0.0032 0.0826 ± 0.005

Values are the mean ± SEM; n=3

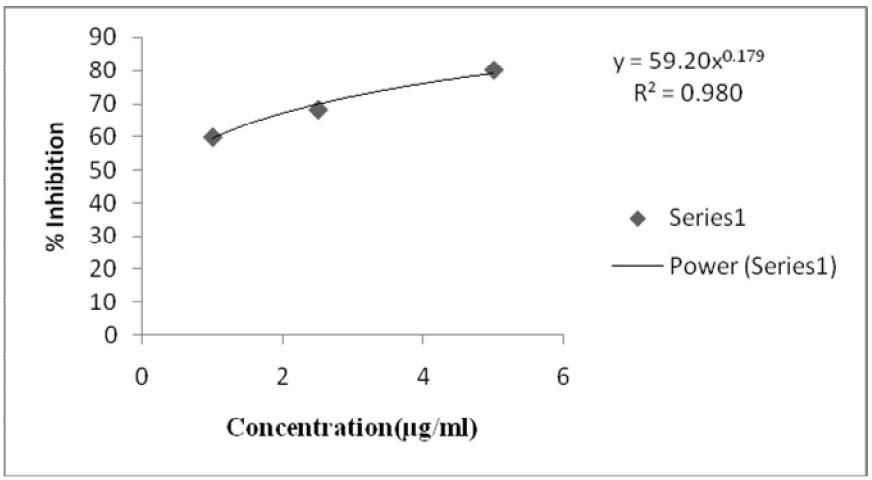

Fig. 1: DPPH antioxidant activity of Gallic acid (standard)

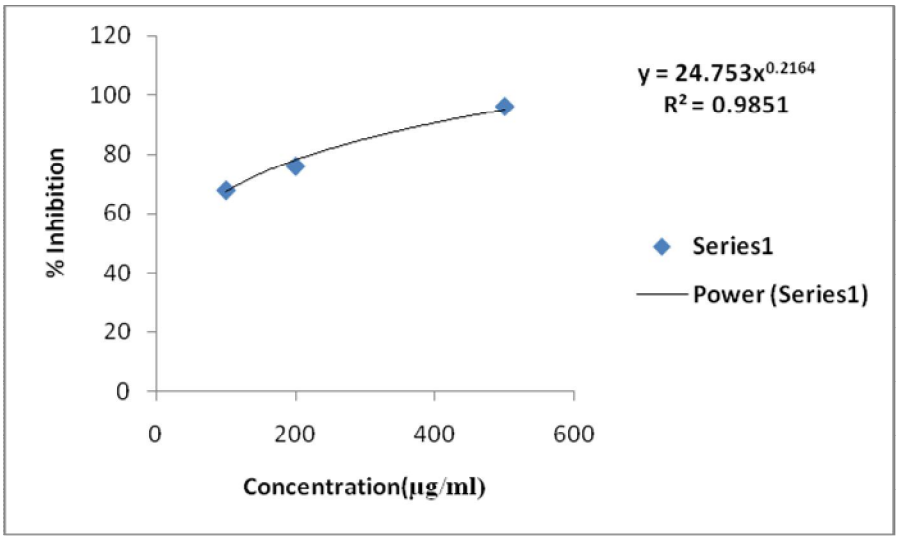

Fig. 2: DPPH antioxidant activity of *Ixoracoccinea*ethanolic leaf extract

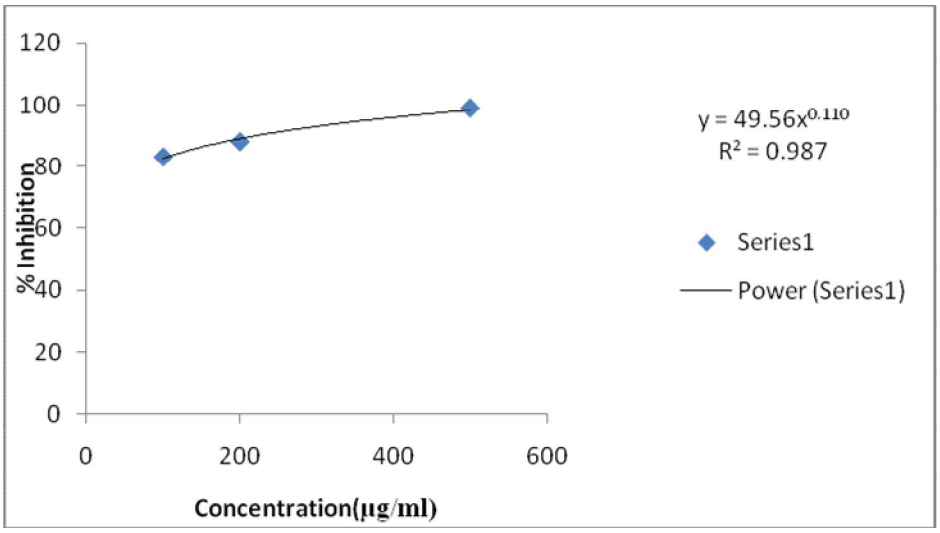

Fig. 3: Nitric oxide antioxidant activity of *Ixoracoccinea* ethanolic leaf extract

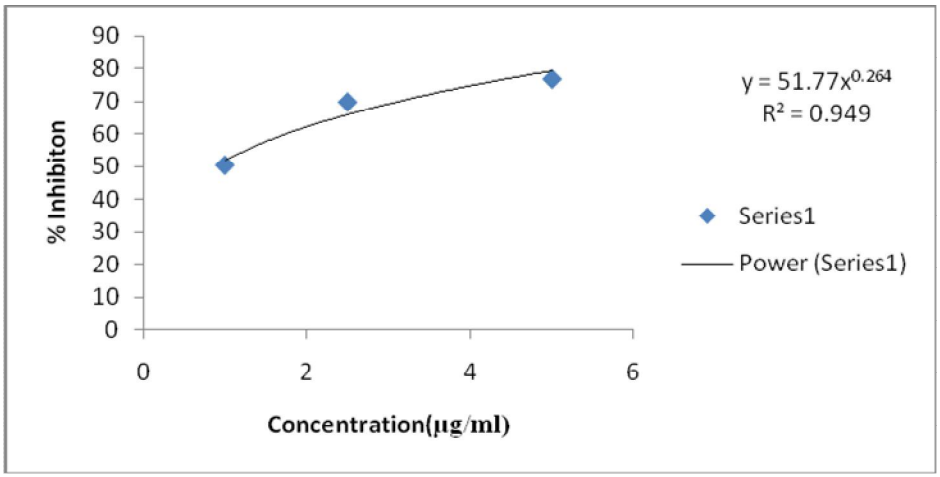

Fig. 4: Nitric oxide antioxidant activity of Gallic acid (standard)

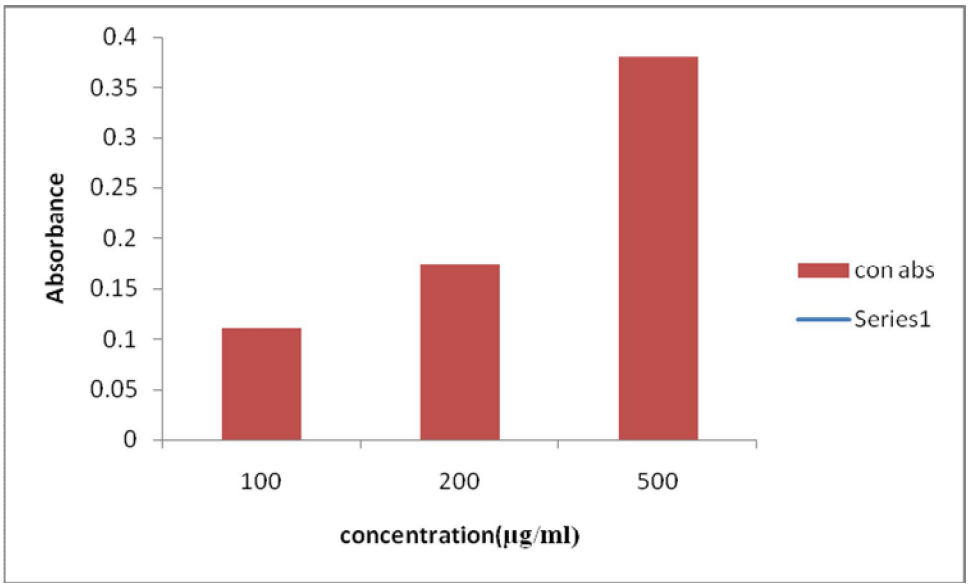

Fig. 5: Reducing power assay of *Ixoracoccinea*ethanolic leaf extract

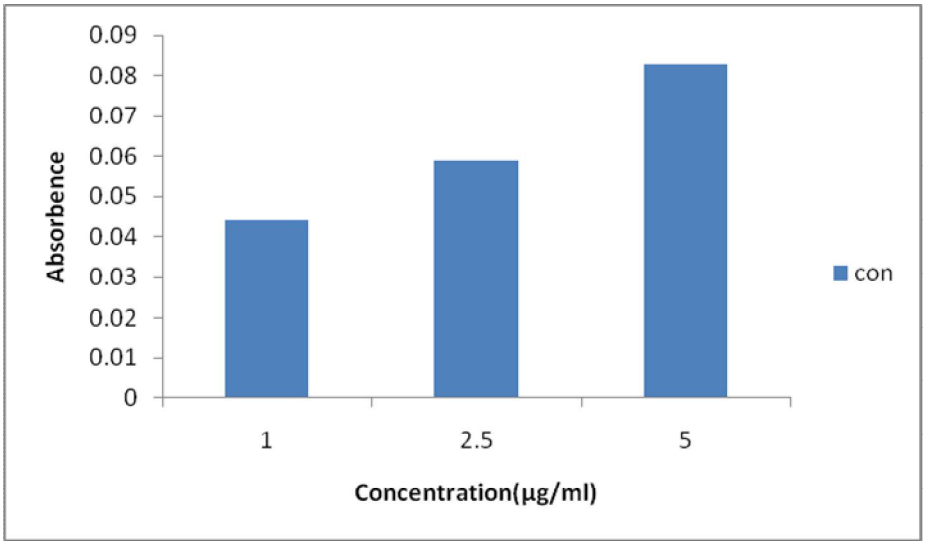

Fig. 6: Reducing power assay of Gallic acid (standard)

RESULTS AND DISCUSSION

DPPH radical scavenging assay

The reduction capability of Dpph radicals was determined by the decrease in its absorbance[2] 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_{50} 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[2]. 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 ixoracoccinealeaf extract reveled 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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