academicJournals

Vol. 7(41), pp. 3071-3075, 3 November, 2013
DOI: 10.5897/JMPR2013.5220
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

In vitro and *in vivo* antioxidant activity of *Ixora coccinea*

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Accepted 10 October, 2013

***Ixora coccinea* Linn. commonly known as Rangon, belongs to the family Rubiaceae. Antioxidant activity of root *of I. coccinea* extract was evaluated in a series of *in vitro* assay involving free radicals. IC_{50} values were determined. Extracts of *I. coccinea* exhibited scavenging effect in concentration dependent manner in all *in vitro* method. IC_{50} of ethyl acetate extract was 48.43, 75.811 and 60.60 µg/ml for 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging, hydrogen peroxide scavenging, nitric oxide scavenging, respectively. Ethyl acetate extract shows good *in vitro* antioxidant activity compared to other extract. The extract was also studied for *in vivo* antioxidant activity by prolongation of haloperidol induced catalepsy. Methanolic extract shows good *in vivo* antioxidant activity compared to other extract.**

Key words: *Ixora coccinea,* DPPH free radical, nitric oxide radical, hydrogen peroxide scavenging, haloperidol induced catalepsy.

INTRODUCTION

Reactive oxygen species (ROS), which consist of free radicals such as superoxide anion ($O_2^{\cdot-}$) and hydroxyl ($HO\cdot$) radicals and non-free radical species such as H_2O_2 and single oxygen ($^1O_2$), are different forms of activated oxygen. ROS are produced by all aerobic organisms and can easily react with most biological molecules including proteins, lipids, lipoproteins and DNA. Thus, ample generation of ROS proceed to a variety of pathophysiological disorders such as arthritis, diabetes, inflammation, cancer and genotoxicity. Therefore, living organisms possess a number of protective mechanisms against the oxidative stress and toxic effects of ROS (Viturro et al., 1999). Antioxidants regulate various oxidative reactions naturally occurring in tissues. Antioxidants can terminate or retard the oxidation process by scavenging free radicals, chelating free catalytic metals and also by acting as electron donors (Shenoy and Shirwaikar, 2002).

Ixora coccinea (Rubiaceae) is a bushy, rounded shrub found in subtropical region of Florida. The plant is grown as ornamental plant in India. It is commonly known as Rangon (Bengali), flame of wood (English) and Bandhaka (Sanscrit). Flowers contain anthocyanins (Krishnamoorthy and Sheshadri, 1962), lupeol (Zacharich et al., 1994), cycloartenol esters, ursolic acid and oleanolic acid (Ragasa et al., 2001), while root contains 9, 11-octadecadienoic acid, myristic acid, quercetin (Kartha, 1967) and leaves contain plastaquinones (Griffiths et al., 1966). Roots show antiinfammatory activity (Seetha et al., 1991; Padmaji et al., 1993). Flowers show cytotoxic (Latha et al., 1988), hepatoprotective (Latha et al., 2003), antimicrobial activity (Annapurna et al., 2004) and leaves shows anti-nociceptive activity (Ratnasooriya and Bashige, 2005). Literature review reveals that no work has been done on antioxidant activity of extracts of root *of I. coccinea*.

MATERIAL AND METHODS

Plant

Roots of *I. coccinea* were collected from Nashik district in May, 2008 and authentified by P. S. N. Rao, joint director, Botanical

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Survey of India, Pune and herbarium specimen deposited as vou.no.ARS-1.

Chemicals

Chemicals used in this study were 2,2-diphenylpicrylhydrazyl (DPPH) obtained from Sigma-Aldrich, India, Nicotinamide adenine dinucleotide (NADH) and sulfanilamide obtained from Himedia, Laboratories Pvt. Ltd., India, obtained from Qualigens Fine Chemicals, Glaxo Smithkline Pharmaceutical Ltd., India, *N*-1-naphthylethylenediamine dihydrochloride, sodium nitroprusside, sodium nitrite, ascorbic acid, tocopherol, Sd Fine Chemicals Ltd, India. All reagents used in the study were of analytical grade.

Preparation of extract

The plant material were air dried in shade, pulverized and extracted and successively extracted with petroleum ether, chloroform, ethyl acetate and methanol in Soxhlet apparatus. The extracts obtained were dried in vacuum oven.

In vitro antioxidant method

DPPH free radical scavenging activity

DPPH scavenging potential of different *I. coccinea* extracts was measured based on scavenging ability of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The method modified by Brand-Williams (1995) was employed to investigate the free radical scavenging activity. Freshly prepared 2 ml DPPH (33 mg/L) solution was thoroughly mixed with 2 ml of different *I. coccinea* extracts. There action mixture was incubated for 1 h at room temperature. Absorbance of the resultant mixture was recorded at 517 nm using ultraviolet–visible spectroscopy (UV-VIS spectrophotometer) (Baheti et al., 2005). The percentage of DPPH scavenging by the extracts and standard compounds were calculated as follows:

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

A_0: absorbance of the control and A_1: absorbance in the presence of the sample of extract and standard. Plotting the graph of % inhibition versus concentration, the IC_{50} was calculated.

Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH, spontaneously produced nitric oxide, which reacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction (Garrat, 1964). Griess Illosvoy reagent was slightly modified using naphthylethylenediamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). Scavengers of nitric oxide compete with oxygen and reduce the production nitric oxide (Marcocci et al., 1994). The reaction mixture (3 ml) containing 2 ml of 10 mM sodium nitroprusside, 0.5 ml of phosphate buffer saline (pH 7.4, 0.01 M) and 0.5 ml of extract was incubated for 150 min at 25°C. Thereafter, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotisation. Then, 1 ml of naphthylethylenediamine dihydrochloride (0.1%) was added and allowed to stand for 30 min in diffused light. The absorbance of the pink coloured chromophore was measured at 540 nm against the corresponding blank solutions. % Inhibition and IC_{50} was calculated in a similar way given in DPPH free radical scavenging activity.

Hydrogen peroxide scavenging activity assay

The ability of the extracts to scavenge hydrogen peroxide was determined according to recently published papers (Nabavi et al., 2009). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extracts (0.1 to 1 mg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. % inhibition and IC_{50} calculated similarly as given in DPPH free radical scavenging activity (Nabavi et al., 2009).

In vivo antioxidant method

Prolongation of haloperidol-induced catalepsy in mice

Haloperidol (1 mg/kg) was injected intraperitoneally (i.p.) to mice (n = 5) pretreated with vehicle (PEG – 0.1 ml, i.p.), petroleum ether extract (PE), chloroform extract (CE), ethyl acetate extract (ETE) and methanol extract (ME) (50 mg/kg, i.p. each). The vehicle or extracts were administered 30 min prior to administration of haloperidol. Vehicle alone was also tested for catalepsy. The duration of catalepsy was measured at 0, 30, 60, 90, 120, 150, 180 min using the Bar test. Both the forepaws of mouse were placed on a horizontal bar raised 3 cm from the table and the time required to remove the forepaws from the bar was recorded as the duration of catalepsy. Between measurements, the animals were returned to their home cages (Ferre et al., 1990).

RESULT AND DISCUSSION

DPPH radical-scavenging activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Ebrahimzadeh et al., 2008). DPPH is a stable nitrogen-centered free radical, the color which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Dehpour et al., 2009). It was found that the radical-scavenging activities of all the extracts increased with increasing concentration (Figure 1). IC_{50} for DPPH radical-scavenging activity were reported in Table 1.

Nitric oxide scavenging activity

Nitric oxide (NO) is a potent pleiotropic mediator of physiological process such as smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities. Although nitric oxide and superoxide radicals are involved in host defense, over production of these

Table 1. Antioxidant profile (IC_{50} values) of extracts of root of *Ixora coccinea*.

Sample	DPPH radical scavenging IC_{50} (µg/ml)	Hydrogen peroxide scavenging IC_{50} (µg/ml)	Nitric oxide scavenging IC_{50} (µg/ml)
Petroleum ether extract (PE)	144.58	108.77	107.59
Chloroform extract (CE)	102.88	102.99	73.08
Ethyl acetate extract (ETE)	48.43	75.811	60.60
Methanol extract (ME)	64.519	91.9	68.44
Ascorbic acid (ASS)	13.86	-	12.59
Tocopherol	-	35.23	-

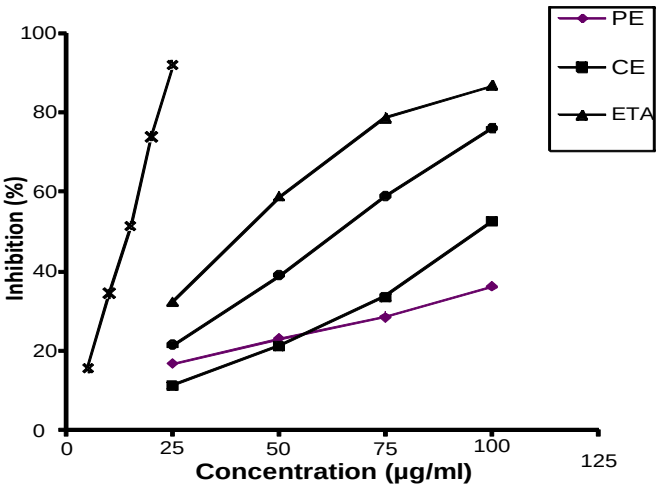

Figure 1. DPPH free radical scavenging activity of extracts of *I. coccinea* roots.

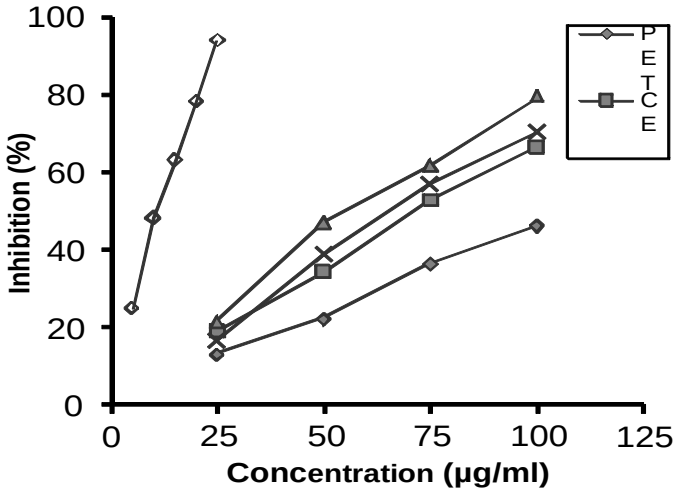

Figure 2. Nitric oxide scavenging activity of extracts of *I. coccinea* roots.

two radicals contributes to the pathogenesis of some inflammatory diseases. Moreover in the pathological conditions, nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules, peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspect of inflammation and tissue damage seen in inflammatory diseases (Kelm et al., 2000). Extracts of *I. coccinea* significantly inhibited nitric oxide in a concentration-dependent manner (Figure 2). The IC_{50} for scavenging of Nitric oxide were given in Table 1. The result indicated that the extract might contain compounds able to inhibit nitric oxide.

Hydrogen peroxide scavenging assay

Scavenging of H_2O_2 by extracts may be attributed to their phenolics which can donate electrons to H_2O_2, thus neutralizing it to water (Ebrahimzadeh et al., 2009). The ability of the extracts to effectively scavenge hydrogen peroxide, determined according to the method of Gulcin (Gulcin et al., 2005), where they are compared with that of tocopherol as standard. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner (Figure 3). The IC_{50} for scavenging of H_2O_2 were given in Table 1. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems (Nabavi et al., 2009).

Prolongation of haloperidol-Induced catalepsy

In vivo antioxidant activity was studied using haloperidol-induced catalepsy in mice. The induction of catalepsy is a phenomenon defined as the long-term maintenance of an animal in an abnormal posture. Metabolism of haloperidol in body takes place by oxidative mechanism. Haloperidol showed catalepsy maximum up to 60 min and then starts to diminish due to oxidation (Munkvad et al., 1968). When haloperidol was combined with antioxidant, antioxidant potentiated haloperidol-induced catalepsy after 60 min. The potentiation of haloperidol-induced catalepsy is due to inhibition of metabolism of haloperidol. Methanolic extract potentiate haloperidol-induced catalepsy (Figure 4). Methanolic extract shows good *in-vivo* antioxidant activity compared to other extracts.

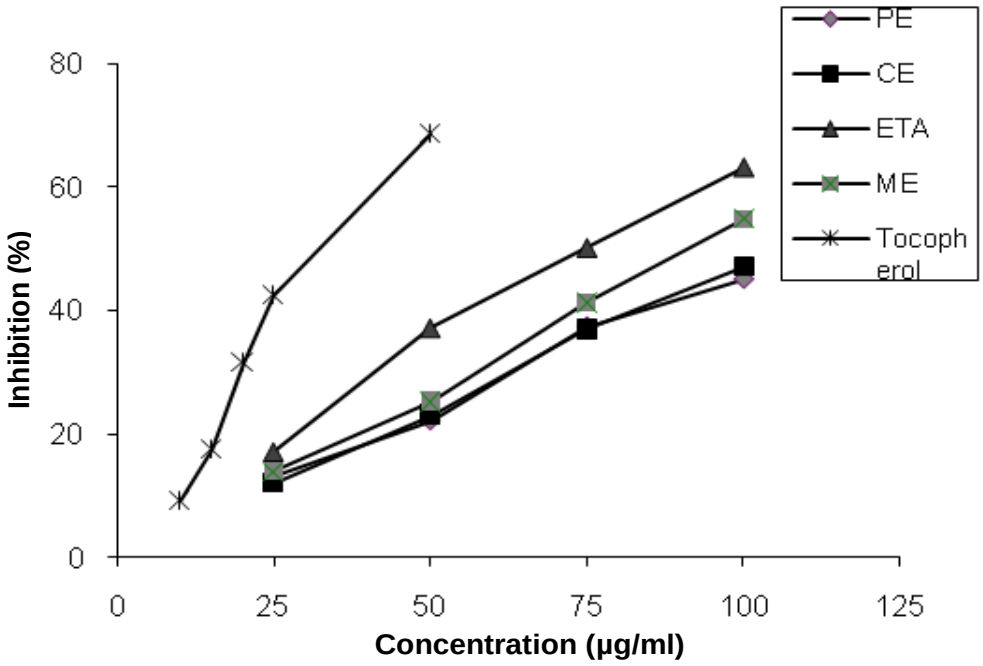

Figure 3. Hydrogen peroxide scavenging activity of extracts of *I. coccinea* roots.

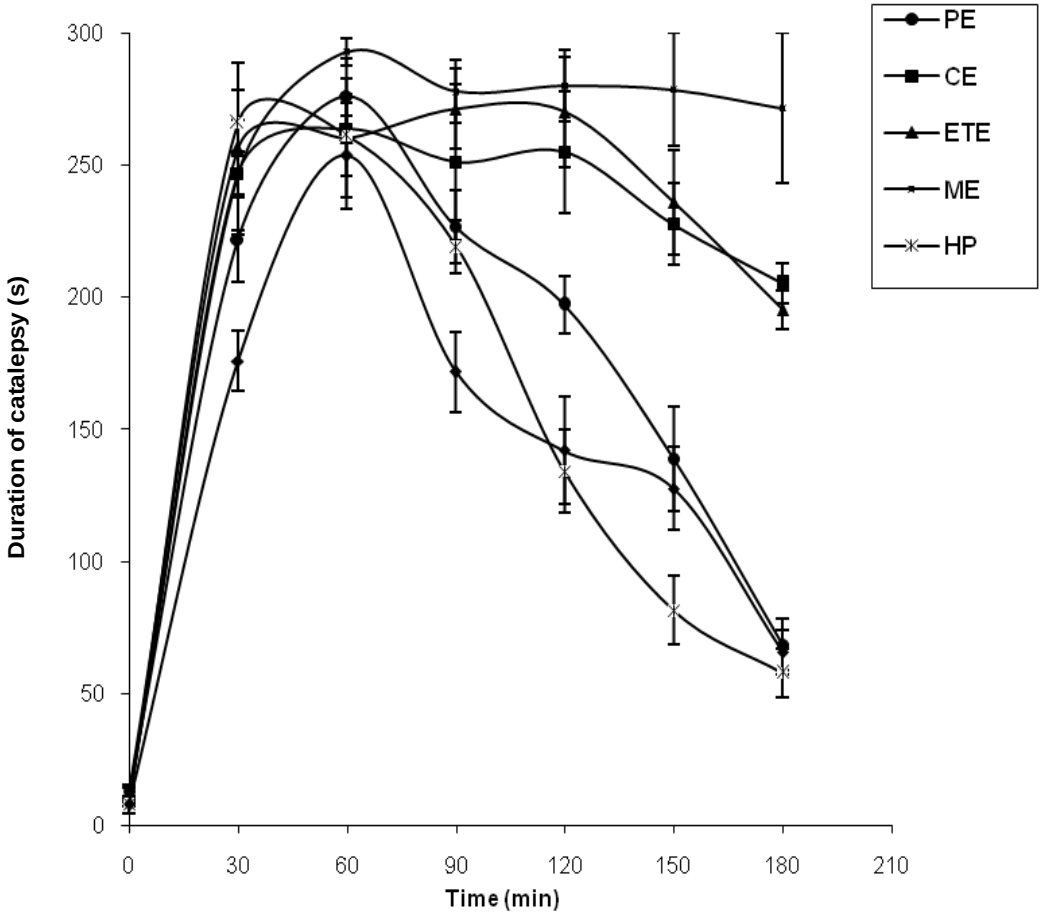

Figure 4. Effect of extracts of *I. coccinea* root of haloperidol-induced catalepsy in mice.

Conclusion

Free radical scavenging effect of *I. coccinea* extracts increases with increasing concentration. In future, work should be done on the isolation and identification of other antioxidant components of *I. coccinea.*

ACKNOWLEDGMENTS

This research was partially supported by a grant from the AICTE, New Delhi

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