ASIAN J. EXP. BIOL. SCI. VOL 2(4) 2011: 595-599

ISSN 0975-5845
AJEBS
http://www.ajebs.com

ORIGINAL ARTICLE

Nitric Oxide Scavenging Activity Study of Ethanolic Extracts of *Ixora coccinea* from Two Different Areas of Kolkata

S. Banerjee*, A. Chanda, A. Ghoshal, R. Debnath, S. Chakraborty, R.Saha, A.Das.
Dept. of Biotechnology, Heritage Institute of Technology, Chowbaga Road, Anandapur, Kolkata-700107.

ABSTRACT

A plant-based diet reduces the risk of development of several chronic diseases. It is a fact that antioxidants contribute to this protection. It would be useful to know the total concentration of electron donating antioxidants in individual plants. The present investigation was based on the assessment of total antioxidant activity from both the flowers and leaves of Ixora coccinea from two different places - an urban and a polluted zone -the institute ground and East Calcutta Wetlands respectively. Antioxidant profile study was done to get an overall idea about the change of the pharmacological property of this plant with respect to environmental pollution. Ethanolic extracts of both dry and fresh leaves and flowers were screened for phytochemical constituents- tannins and flavonoids, were estimated. Samples were investigated to evaluate free-radical scavenging effect by nitric oxide scavenging activity. From the above results, it is evident that both the leaves and flowers of Ixora coccinea, obtained from East Calcutta Wetlands, contain significantly higher concentrations of the antioxidants, flavonoids and tannins than the institute samples.

Keywords: *Ixora coccinea*, *Antioxidant activity, nitric oxide scavenging activity, tannins, flavonoids*

INTRODUCTION

Ixora coccinea is an evergreen flowering shrub, native to the tropical regions of Asia. It belongs to the family Rubiaceae. *I. coccinea* is a dense, multi-branched shrub [1]. It has several medicinal properties - chemoprotective, cytotoxic and anti-tumour activity [2], antimicrobial activity [3], and antinociceptive activity [4]. From ancient times different plant parts have been used in treatment of diarrhea, dysentery, leucorrhoea, dysmenorrhoea, hemoptysis and catarrhal bronchitis [5]. The flower extract has been found to contain triterpenoids, tannins and flavonoids which infer certain medicinal properties upon them[6]. Antioxidants act as reducing agents which prevent oxidative damage to cellular components such as DNA, proteins and lipids by reactive oxygen species (ROS) produced in cells. Reactive oxygen species including superoxide (O_2^-), Hydrogen peroxide (H_2O_2), nitric oxide (NO) and hydroxyl (OH^-) exert oxidative stress in the cells of human body rendering each cell to face about 10,000 oxidative hits per second [7-8]. Ursolic acid is the main triterpene isolated from *Ixora coccinea* flower [9]. It has been found to have significant antioxidant activity [10]. Tannins are polyphenolic compounds which are capable of cross-linking with proteins, forming stable water insoluble copolymers. Tannins have been shown to have antioxidant potential as well as antiviral, antibacterial and antiparasitic properties [11]. Flavonoids are water soluble polyphenolic compounds derived from flavones. Flavonoids have been shown to reduce risk of death in coronary heart disease and cardiovascular disease [12]. *In vitro* studies of flavonoids have displayed anti-allergic, anti-inflammatory [13], anti-microbial [14] and anti-cancer activities [15]. Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in living organisms as it acts as scavengers of singlet oxygen and free radicals [16-17]. NO is a free radical which is an effective inhibitor of several physiological processes such as smooth muscle relaxation and neuronal signaling [18].

The present study aims to evaluate the expression of tannins, flavonoids and nitric oxide scavenging activity in the leaf and flower of *I.coccinea* collected from two different locations and to show their variation depending on the environment to which they are exposed. The first location is the institute garden, which constitutes a relatively

unpolluted urban area, where the soil is regularly maintained. The second is the East Calcutta Wetlands (EKW), which acts as the waste recycling region of the city and its surroundings. It may be classified as a polluted area due to the release of high concentration of toxic heavy metals from industries, tanneries, and agriculture as well as health sectors [19]. Many industries, especially electroplating, battery and plastic manufacturing units release heavy metals such as Cadmium and Zinc in wastewater [20], resulting in high oxidative stress.

In the study presented here, the antioxidant activity of ethanolic extracts of both the leaves and flowers of *Ixora coccinea* were determined by nitric oxide scavenging assay, as well as by flavonoid and tannin estimation.

MATERIALS & METHODS

Sample preparation: Both the leaves and flowers of *Ixora coccinea* were collected and washed thoroughly under running water. 2gms of both leaves and flowers (fresh and dried) were weighed and crushed in ethanol. It was kept at 4ºC for 48 hours. Subsequently, it was filtered and centrifuged at 10,000rpm for 10mins. Supernatant was further diluted with ethanol as per the assay.

Nitric oxide scavenging assay: Nitric oxide scavenging activity was measured spectrophotometrically [21]. Extract, prepared in ethanol, was added to different test-tubes in varying concentrations (0.25, 0.5, 1, 1.5, 2, 2.5 mg/ml). Sodium nitroprusside (5mM) in phosphate buffer was added to each test tube to make volume up to 1.5ml. Solutions were incubated at 25ºC for 30 minutes. Thereafter, 1.5ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added to each test tube. The absorbance was measured, immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard.

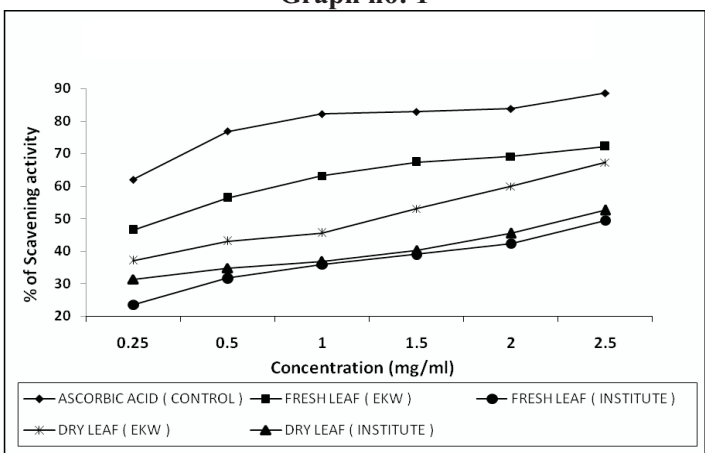
Aluminium Chloride colorimetric assay (Flavonoids estimation): Total flavonoids were estimated by a colorimetric assay by aluminium chloride method [22]. Few modifications were done as per the requirement of the test. 500µl of suitably diluted sample was taken to which 300µl of 5% $NaNO_2$ was added immediately. 250µl of 10% $AlCl_3$ was added after 5 minutes and 1ml of 1(M) NaOH was added after another 1 minute. The absorbance was noted at 510nm and concentration was estimated with respect to Quercetin as standard.

Tannin estimation: Tannins were estimated spectrophotometrically [23], with minimal modifications. 0.5 ml of suitably diluted extract was taken in a test tube and volume was made up to 2.5 ml with distilled water. 0.25 ml of 1:19 diluted Folin Ciocalteau reagent and 0.5 ml of 20% sodium carbonate solution were added. The solution was kept for 30 minutes at room temperature. Subsequently, absorbance was measured at 775nm and concentration was estimated with respect to tannic acid as standard.

RESULTS AND DISCUSSION

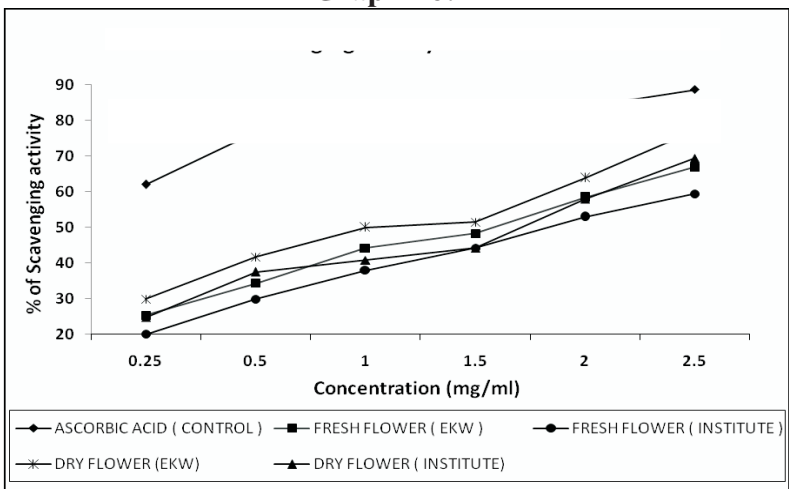
Extracts of *Ixora coccinea* revealed the significant presence of antioxidative agents like flavonoids and tannins. Nitric Oxide (NO) scavenging assay is based on the scavenging ability of the extracts as well as ascorbic acid, which is used as standard. The scavenging of NO was found to increase in dose dependent manner. Maximum inhibition of NO was observed in the extracts of highest concentration (2.5mg/ml) for both the samples. At this maximum concentration, inhibition was found to be 88.66% for ascorbic acid, which serves as the standard. For dry leaf extract, inhibition was found to be higher 67.28% for the sample from East Calcutta Wetlands, and similarly it is 52.52% from the sample from the institute garden. Similar observations were found for fresh leaf extract, which were 49.43% and 72.18% in the samples from the institute garden and East Calcutta Wetlands respectively as shown in graph no 1. For dry flower extract, inhibition was found to be 59.43% and 66.93% in the samples from the institute garden and East Calcutta Wetlands respectively. Similar observations were found for fresh flower extract, which were 69.45% and 77.24% in the samples from the institute garden and East Calcutta Wetlands respectively as shown in graph no 2.

Graph no: 1



Graph no: 1
Nitric oxide scavenging activity in Ixora coccinea flowers

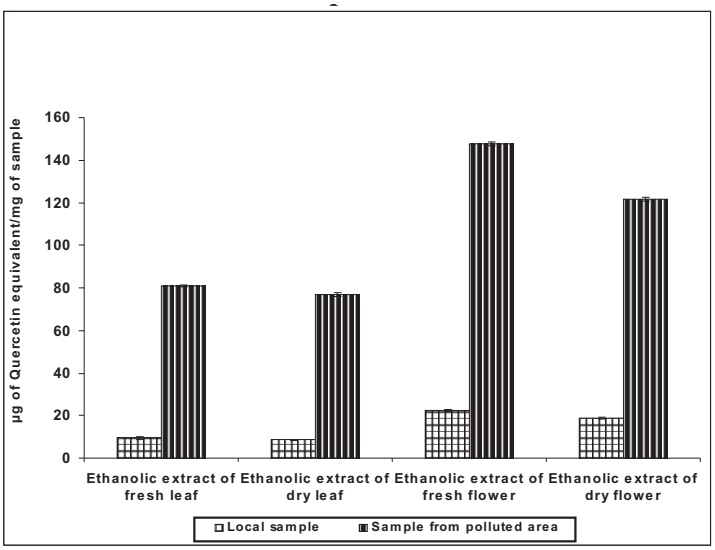
Graph no: 2



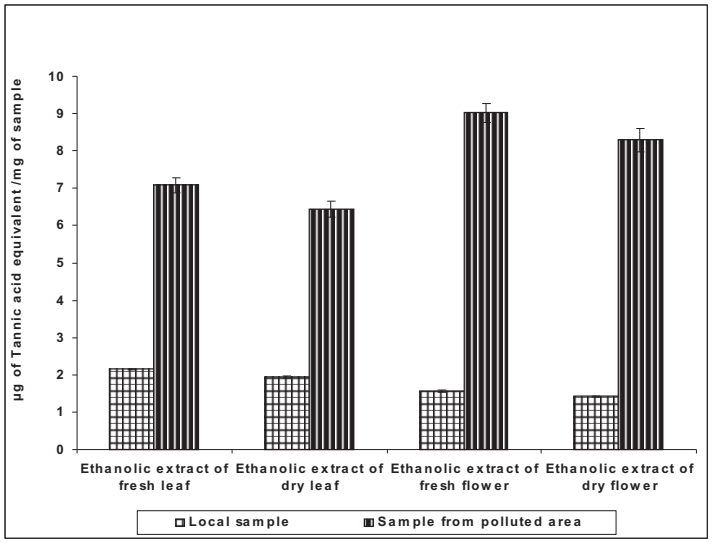
Graph no: 2
Nitric oxide scavenging activity in Ixora coccinea flowers

Colorimetric determination of flavonoids in plants, utilizing $AlCl_3$ reagent, is an established method to quantify total flavonoid content. For dry leaf extract, total flavonoids were found to be 8.55 and 76.76 µgs of Quercetin equivalent/mg of sample from the institute garden and East Calcutta Wetlands respectively. For fresh leaf extract, total flavonoids were found to be 9.42 and 80.88 µgs/mg of sample from the institute garden and East Calcutta Wetlands respectively. For dry flower extract, total flavonoids were found to be 18.75 and 121.74 µgs/mg of sample from the institute garden and East Calcutta Wetlands respectively. For fresh flower extract, total flavonoids were found to be 22.41 and 147.62 µgs/mg of samples from the institute garden and East Calcutta Wetlands respectively as shown in graph no: 3

Estimation of total tannin is based on oxidation of molecules which contain phenolic hydroxyl groups. For dry leaf extract, total tannins were found to be 1.94 and 6.44 µgs/mg of samples from the institute garden and East Calcutta Wetlands respectively. For fresh leaf extract, total tannins were found to be 2.15 and 7.08 µgs of Tannic acid equivalent/10 mgs of samples from the institute garden and East Calcutta Wetlands respectively. For dry flower extract, total flavonoids were found to be 1.42 and 8.29 µgs/mg of samples from the institute garden and East Calcutta Wetlands respectively. For fresh flower extract, total tannins were found to be 1.56 and 9.02 µgs/mg of samples from the institute garden and East Calcutta Wetlands respectively as shown in graph no: 4



Graph No. 3
Total flavonids in flowers and leaves of Lxora coccinea



Graph No. 4
Total Tannin in flowers and leaves of Lxora coccinea

From the above results, it is evident that both the leaves and flowers of *Ixora coccinea* obtained from East Calcutta Wetlands contain significantly higher concentration of the antioxidants activity and higher amounts of flavonoids and tannins than the institute samples. It may be due to the stress, induced by the toxic heavy metals released abundantly by the neighboring industries, on the plants growing in the East Calcutta Wetlands. A direct relationship between heavy metal stress and increased antioxidant production mechanism has been found in plants in several cases [24-27]. It can also be added from the results that ethanolic extracts of *Ixora coccinea* contain antioxidant activity which may be responsible for the diverse uses of the plant for treatment of various ailments.

ACKNOWLEDGMENTS

The authors wish to acknowledge the authorities of Heritage Institute of Technology for providing necessary technical support for this work.

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Corresponding Author: Soma Banerjee Dept. of Biotechnolog y, Heritage Institute of Technology, Chowbaga Road, Ananadapur, Calcutta-700107. Email:soma.banerjee@heritageit.edu