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EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF *ERYTHRINA INDICA* LEAVES

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ABSTRACT

The antioxidant activity of methanol extract of the leaves of *Erythrina indica* was evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and Hydrogen peroxide scavenging assay methods. Ascorbic acid was used as a reference standard. The extract revealed significant activities in both antioxidant assays compared to the reference standard in a concentration dependent manner.The results suggest that the antioxidant properties of the extract may be due to the presence of flavonoids and related polyphenolic compounds, as reported in previously published literatures.

KEYWORDS: ***Erythrina indica,*** **Antioxidant activity, DPPH, Hydrogen peroxide.**

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INTRODUCTION

Free radicals are highly unstable chemical entities of atoms or molecules that possess an unpaired electron in their outer most orbital. Therefore these radicals are highly reactive and can interact with other molecules by giving out or accepting single electron [1,2]. These radicals are produced on a continuous basis in cells as by-products from metabolism or during other cellular activities. Around 5% of the oxygen that is inhaled is converted to reactive oxygen species (ROS). ROS are generated intracellularly by several sources, including mitochondria; the primary sources of ROS involved in receptor-mediated signalling cascades are plasma membrane oxidases, preferentially NADPH oxidases, with a rapid kinetics of activation and inactivation [3]. Oxidative stress results when the balance between ROS production and antioxidant defences is lost, which leads to modifications of lipid, protein and DNA. Deregulation of the cellular functions occurs due to oxidative modifications of macromolecules. This leads to conditions like inflammation, arthritis, cataract, atherosclerosis, cardiovascular diseases, cancer as well as ageing. These degenerative disease conditions can be easily and effectively delayed by preventing or decreasing the oxidative damage. Thus now, the use of antioxidants is increasingly being recognized as one of the ways to prevent these oxidative damages thus preventing or at least delaying the related diseases [4-6].

Figure 1
Erythrina indica Lam.

Erythrina indica Lam. (Fig. 1) belongs to family Fabaceae and is popularly known by various other names like Indian coral tree or Tiger's claw or Sunshine tree [7]. It is a medium sized thorny tree with deep tap roots and strong wooded stem. Leaves are shiny, smooth, trifoliate, and alternate with spiny petiole and rachis. They show pinnate venation with entire margins and are oval in shape [7-11]. A thorough literature survey reveals that the leaves have been tested for the antioxidant property using their ethanol extract, by ferric thiocyanate and thiobarbituric acid methods [12-13]. Also, Saraswathy A et al has reported *in-vitro* antioxidant activity and heavy metal analysis of stem bark of *Erythrina indica*.Lam. In the present paper, we report the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and Hydrogen peroxide scavenging assay of the methanol extract. To determine the free radical scavenging activity of *E. Indica* leaves extract, a method based on reduction of methanolic solution of colored free radical DPPH was used [14].

MATERIALS AND METHODS

- ***Collection of plant materials***

Fresh leaves of *E. indica* Lam were collected from in and around Navi Mumbai, identified and authenticated by Dr J. Jayanthi, Botanical Survey of India, Pune (Specimen no. RUPERVA1 dated 31/07/2015). The leaves were cleaned under water, shade dried, powdered and stored in a cool place till further use.

- ***Chemicals and reagent***

DPPH (1,1-diphenyl-2-picryl hydrazyl) was procured from Sigma Aldrich Laboratories, USA. Hydrogen peroxide solution (25 mmol/L), Phosphate buffer (pH 7.4), ascorbic acid etc. used in the study were of AR grade.

- ***Instrumentation***

JASCO V 550 UV –VIS Spectrophotometer was employed for all spectroscopic measurements using a pair of matched quartz cells. Other common glassware's were the basic laboratory apparatus of Borosil® make. The glasswares were soaked in chromic acid for 3 days, washed with tap water followed by rinsing with distilled water and finally dried in the oven.

- ***Preparation of leaf extract***

The shade dried powdered leaves were extracted by the percolation method using 90% methanol. The resultant extract was then evaporated and dried under reduced pressure. The dried residue was reconstituted with 90% methanol to get final concentration (2mg/ml).

• ***DPPH free radical scavenging activity***

About 3 ml of methanol and 50µl of DPPH reagent (2mg/ml) were added to set of test tubes. The initial absorbance was measured at 516nm. To these test tubes, different volumes (10-100µl) of *E. indica* extract was added. Reaction time was set at 4 min, after which the absorbance of the solutions were again measured and the % reduction in their absorbance values were calculated. Similarly, ascorbic acid (0.5mg/ml) was added in the range of 10-25 µl and absorbance was measured at 516 nm. A blank solution containing 50µl of DPPH in 3ml of methanol was maintained throughout the experiment and absorbance recorded at every 30 min up to 3h. There was no change in the absorbance showing the stability of free radical [15]. Results of the study are presented in Table 1. A graph of % inhibition versus concentration was plotted (Fig. 2).

• ***Hydrogen Peroxide Scavenging assay: (16, 17)***

A solution of hydrogen peroxide (25mmol/l) was prepared in phosphate buffer (pH 7.4). The test extract was diluted to different concentrations (1-5%v/v) and was added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was taken after 10 min against a blank solution having phosphate buffer in absence of hydrogen peroxide. For each concentration, a separate blank sample was used. The percentage scavenging activity of hydrogen peroxide by sample and standard formulations were calculated using the following formula:

$$\%\ Scavenging\ activity = \frac{[Abs(control) - Abs(standard)]}{Abs(control)} \times 100$$

Results of the study are presented in Table 2. A graph of % inhibition versus concentration was plotted (Fig. 3).

RESULTS AND DISCUSSION

The DPPH radical scavenging assay is the most widely used methods for screening antioxidant activity of plant extracts, since it can detect activity at low concentrations. From the above results it was observed that free radicals were scavenged by the test compounds in a dose dependent manner. The decrease in absorbance of the DPPH caused by the methanol extract was due to the scavenging of the radical by hydrogen donation (Table 1, Fig. 3). The extract exhibited strong DPPH radical scavenging activity (IC_{50} 39.86 mg/ml) whereas that of the standard, ascorbic acid was 22.22mg/ml (Fig.2).The extract also scavenged hydrogen peroxide in a concentration-dependent manner. The extract demonstrated strong H_2O_2 scavenging activity (IC_{50} 3.81 mg/ml) (Table 2, Fig. 5) whereas that of the standard, ascorbic acid was 0.52 mg/ml (Fig. 4).

Table 1

DPPH scavenging activity of the methanol extract of E. indica leaves

Concentration (µg/ml)	% inhibition ± standard deviation
7.01	8.023
12.99	16.88
26.23	34.89
50.89	63.57
64.78	80.33

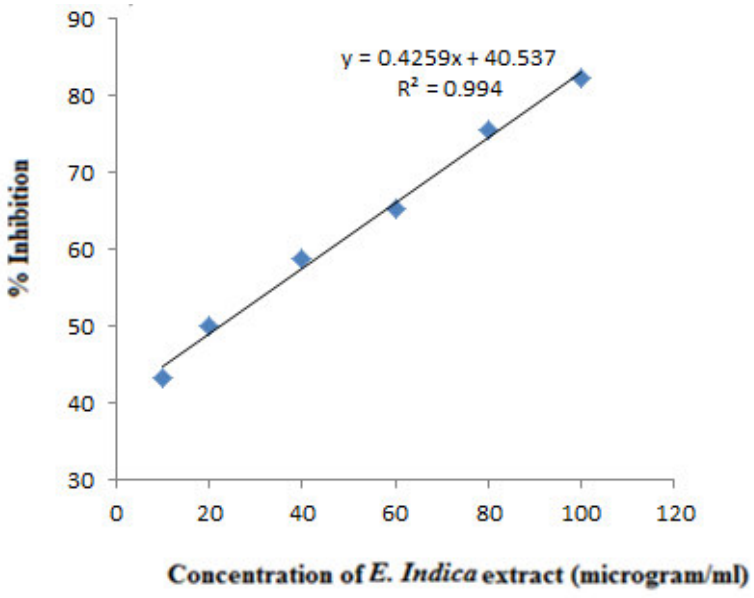

Figure 1

Antioxidant activity of E. indica extract by DPPH assay method (Ascorbic acid)

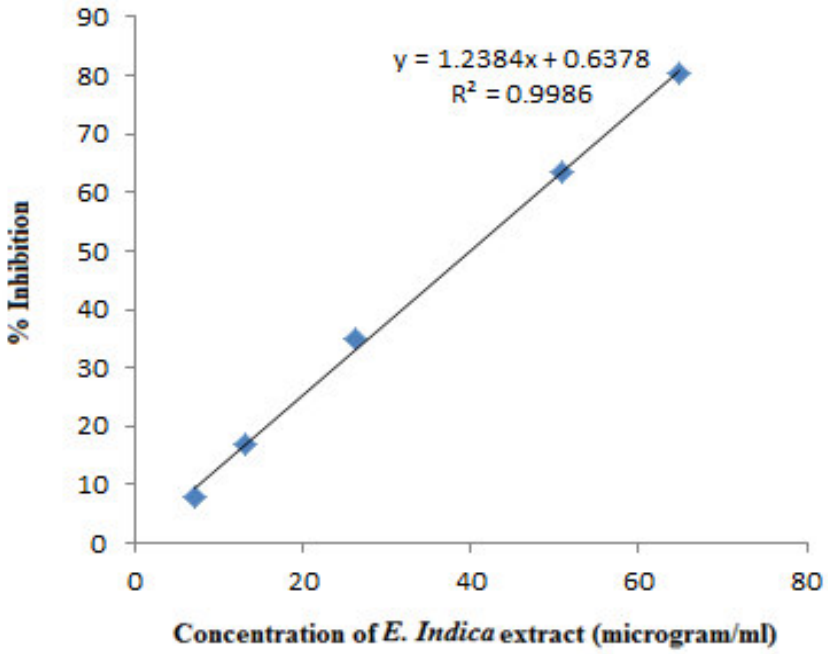

Figure.3
Antioxidant activity of E. indica extract by DPPH assay method

Table 2
Hydrogen peroxide scavenging activity of the methanol extract of *E. Indica* leaves

Concentration (%) v/v	% scavenging activity
1	20.69
2	34.09
3	42.62
4	51.14
5	61.48

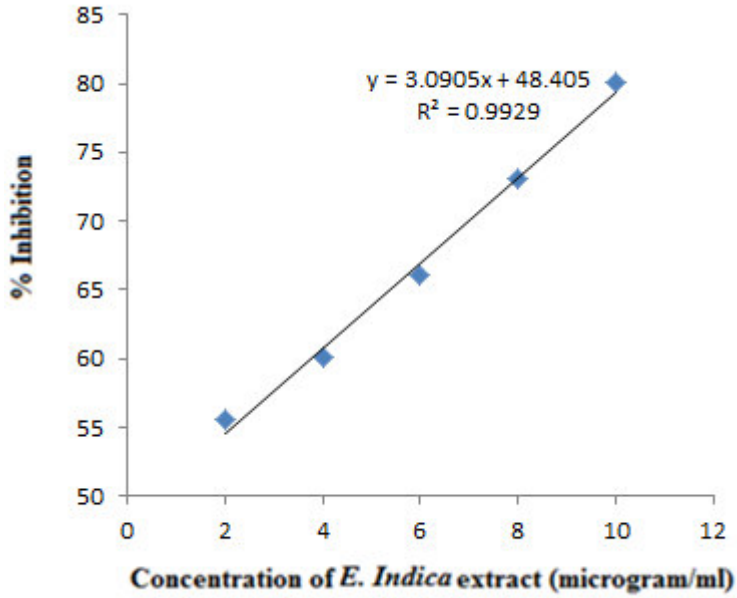

Figure.4
Antioxidant activity of E. indica extract by Hydrogen peroxide assay method (Ascorbic acid)

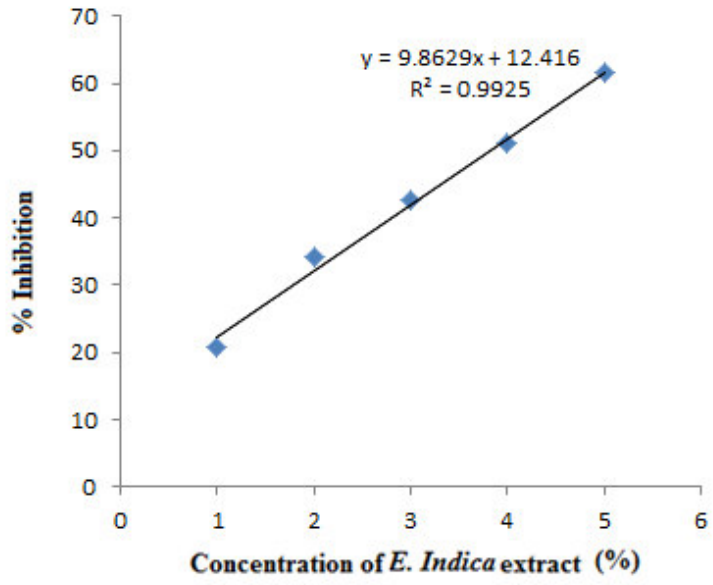

Figure.5
Antioxidant activity of E. indica extract by Hydrogen peroxide assay method

Results of both DPPH and H_2O_2 scavenging activity showed that scavenging property increases with the increased concentration of the extract.

CONCLUSION

E. indica has been reported to possess several medicinal activities in the traditional systems of medicine including antioxidant property, antidiabetic action, antidiarrheal property etc. Antioxidants are always believed to be helpful in protecting our body from the harmful effects of free radicals generated from the biochemical reactions. In the present study, the methanol extract showed good free radical scavenging activity in both the models studied. Thus, we may consider that the leaves of this plant can be used as an efficient natural antioxidant. The methanol extract of *E. indica* leaves have been reported to flavonoids and related polyphenolic compounds by different authors. This study further supports the potential antioxidant activity of the plant.

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CONFLICT OF INTEREST

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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