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In vitro antioxidant activity of methanolic extract of *Erythrina indica*

[1]Komal Moon*, [2]Parimal Katolkar, [1]S. S. Khadabadi

[1]Govt. College of Pharmacy, Kathora Naka, Amravati (M.S.), INDIA
[2]Manoharbhai Patel Institute of Pharmacy, Gondia. (M.S.), INDIA

Abstract

The present study aimed to explore antioxidant potential of bark of Erythrina indica (Leguminoseae). The antioxidant activity of the methanolic extracts of the Erythrina indica was evaluated in-vitro by various Experimental Parameters such as DPPH free radical scarvenging activity, reducing power and nitric oxide scarvenging activity, total phenolic content were also determined. Result showed that Erythrina indica displayed potent antioxidant.

Keywords: Erythrina indica, (DPPH) 1, 1-diphenyl-2-picryl-hydrazyl, Reducing Power Method, Nitric oxide radical scarvenging activity.

INTRODUCTION

Oxidative stress is believed to be a primary factor in various diseases as well as in the normal process of aging [1, 2]. Free radicals and reactive oxygen species (ROS) are well known inducers of cellular and tissue pathogenesis leading to several human diseases such as cancer, inflammatory disorders, atherosclerosis and cardiovascular diseases. Cardiovascular diseases are the most common cause of death in the industrialized countries. The beneficial effects of phytochemicals are associated with a multitude of biological activities, including antioxidant and free radical scavenging properties [3]. Reactive oxygen species includes free radical such as superoxide anion radicals (O_2) hydroxy radical (OH), non free radicals such as H_2O_2 and singlet oxygen ($^1O_2$) along with various form of oxygen. They involved in various physicochemical parameter and diseases such as aging. Several studies reported that plant have antioxidant properties. Butyl hydroxyl anisol (BHA) and butyl hydroxyl toluene (BHT) are most commonly used antioxidant [4]. Antioxidants help organism to deals with oxidative stress which is caused by the free radical damage [5].

Erythrina indica tree reaches 18 m height, bark thin, smooth, grey, and armed with small conical dark coloured prickles. Leaves 15-30 cm long, deciduous, petioles 10-15 cm long unarmed readily disarticulating [6]. Found tropic and sub-tropic region. Also found wild in deciduous forests throughout India and in Andaman and Nicobar Islands [7]. Bark used medicinally as febrifuge and anti-bilious. Bark also used in dysentery. Leaves are used to improve appetite and urinary discharge inflammation [6]. Methanol extract of Erythrina indica leaves shows antiulcer activity in pylorus ligated and indomethacin induced ulceration in the albino rats [8]. The aqueous leaf extract of Sri Lankan Erythrina indica showed potent sedative activity but no analgesic effects [9]. Traditionally it is used for liver trouble, joint pain, dysentery, laxative, diuretic, convulsion and as anthelmintic [8, 10, 11]. Its powdered bark traditionally used for rheumatism, itching, fever, asthama and leprosy [7, 8]. From the stem bark of Erythrina indica two new isoflavone derivatives named indicanines D and E together with 11 known compounds including one cinnamate (erythrinassinate B), two phytosterols (stigmasterol and its 3-O-β-Dglucopyranoside), six isoflavones (genistein, wighteone, alpinumisoflavone, dimethylalpinumisoflavone, 8-prenyl erythrinin C and erysenegalensein E), two pentacyclic triterpenes (oleanolic acid and erythrodiol) [12]. From root bark of Erythrina indica 3-phenylcoumarin metabolite, name dindicanine B, and a new isfloavone derivative, name dindicanine C, were isolated [13]. The prenylated flavones glycoside 5,7,4-trihydroxy-3-methoxy-8-C-prenylflavone 7-O-β-D-glucopyranosyl-(1-3)-α-L-arabinopyranoside was isolated from the seeds of erythrina indica [14]

The purpose of the present study was to evaluate in vitro antioxidant activity of Erythrina indica.

MATERIALS AND METHODS

Plant Material: The bark of Erythrina indica was collected from Nagpur. The plant was authenticated from Department of Botany, Rashtrasant Tukadoji Maharj Nagpur University, Nagpur.

Extraction

The collected material was subjected to defatting with petroleum ether in Soxhlet apparatus followed by extraction with methanol. The methanolic extract was subjected to preliminary phytochemical screening.

Chemicals

1, 1-diphenyl-2-picryl-hydrazyl (DPPH), Nitric oxide, Butylated hydroxyl anisol (BHA), Griess reagent, Sod. Nitroprusside, Folin-Ciocalten reagent.
These chemicals were purchased from Merck.

DPPH free radical scarvenging activity of Methanolic Extract of Erythrina indica [15]

The method was employed by Blois. One ml methanolic extract of Erythrina indica and reference compound in various concentration (50,100, 150, 200, 250µg/ml) was added to one ml of 0.1 mM solution of DPPH in methanol. After 30 min., absorbance was measured at 517 nm using UV Spectrophotometer (UV 1700). A 0.01 mM solution of DPPH in methanol was used as control whereas BHA was used as reference material. All tests were performed in triplicate. Percent inhibition was calculated (equation 1)

Reducing Power Method [16]

The reducing power of methanolic extract was evaluated according to the method of Oyaizu et al 1986. Various concentrations of methanolic extracts of Erythrina indica (50,100, 150, 200, 250µg/ml) were taken 1.0 ml of test sample was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1 % potassium ferricynide. The mixture was made homogeneous and incubated at 500C for 20 min; aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture, which was then centrifuged at 1500 rpm for 10 min (2.5 ml) and finally freshly prepared FeCl3 solution (0.5 ml, 1%) was added to this and mixed uniformly. The absorbance of supernatant was measured at 700 nm. Increased absorbance of reaction mixture indicated increased reducing power. Ascorbic acid was used as standard.

Nitric oxide radical scavenging activity [17]

Nitric oxide generated from sod. Nitroprusside in aqueous solution at physiological pH interacted with oxygen to produce nitrite ion which were measured using the Griess reaction. Three ml of 10 mM sod. Nitroprusside in phosphate buffer was added to two ml methanolic extract and reference compound in different concentration (20, 40, 60, 80 and 100 µg/m). The resulting solution was then incubated at 25o C for 60 min. A similar procedure was repeated with Methanol as blank which served as control. To 5ml of incubated sample, 5 ml Griess reagent (1% Sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H3PO4) was added. The absorbance of the Chromophore formed was measured using spectrophotometer (UV 1700) at 546 nm. All tests were performed in triplicate. Percentage inhibition of the nitric oxide generated was measured by comprising the absorbance value of control and test preparation (equation 1). Curcumin was used as reference material.

Amount of total phenolic compound [18]

Total soluble phenolic compound present in methanolic extract of Erythrina indica were determined by the Folin- Ciocalteu reagent according to the method suggested by Slinkard and Singleton. To 0.1 ml extract, 1ml of folin ciocalteu reagent was added, the mixture shaken for 2 hrs at room temp. And absorbance was measured using a spectrophotometer (UV 1700) at 760 nm. All tests were performed in triplicate. The concentration of total phenolic compound in sample was determined as µg pyrocatechol equivalents using following equation obtained from standard pyrocatechol graph.

$$Absorbance = 0.001 \times pyrocatechol\ (\mu g) + 0.0033$$

Statistical analysis

Data are mean ± SD of three measurement statistical analyses was performed by student t-test and by ANOVO. $P < 0.05$ was regarded as significant.

$$\%\ inhibition = [(Control\ Absorbance - Test\ Absorbance)/Control\ Absorbance] \times 100 \quad (equation\ 1)$$

RESULTS AND DISCUSSION

DPPH free radical scarvenging activity

The DPPH radical is considered to be model lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid autoxidation. The scavenging effects of methanolic extracts of Erythrina indica and BHA on the DPPH radical are illustrated in Table. 1. Methanolic extract had significant scavenging effects on the DPPH radical, which increased with increasing concentration from 50-250 µg/ml. The scavenging effect of methanolic extract was lower than that of BHA.

Reducing power method

The reducing power by methanolic extract of Erythrina indica is shown in Table.2. The antioxidant activity of methanolic extract was less than Ascorbic acid, which was used as a reference compound.

Table -1: DPPH free radical scavenging activity of methanolic extracts of *Erythrina indica* and butylated hydroxyl anisole

Concentration µg/ml	% inhibition	
	Methanolic Extract of *Erythrina indica*	BHA
50	15.49 ± 2.37*	36.17 ± 4.96
100	38.58 ± 5.19*	62.42 ± 3.52
150	44.44 ± 2.91*	70.15 ± 5.12
200	57.55 ± 2.25*	75.28 ± 5.52
250	69.39 ± 4.62	81.59 ± 4.45

Data are the means ±SD of three measurement p< 0.05 compared to control*

Table -2: Antioxidant Activity of methanolic extract of *Erythrina indica* in Reducing Power Method

Concentration µg/ml	% reducing power	
	Methanolic Extract of *Erythrina indica*	Ascorbic acid
50	18.17 ± 1.46*	29.99 ± 3.22
100	36.15 ± 3.19*	44.09 ± 2.52
150	54.44 ± 2.19*	60.00 ± 4.22
200	67.55 ± 2.12*	75.87 ± 4.87
250	79.39 ± 3.13	93.44 ± 4.90

Data are the means ±SD of three measurement p< 0.05 compared to control*

Nitric oxide radical scavenging activity

The percent inhibition of nitric oxide generation by methanolic extract of Erythrina indica is shown in Table.3. The antioxidant activity of methanolic extract was less than curcumin, which was used as a reference compound.

Amount of total phenolic compound

The Methanolic extract was found to have 40 µg pyrocatechol equivalent of phenol respectively.

Table -3: Nitric oxide radical scavenging activity of methanolic extract of *Erythrina indica* and curcumin

Concentration µg/ml	% Inhibition	
	Methanolic Extract of *Erythrina indica*	Curcumin
20	30.52 ± 1.91*	50.12 ± 3.12
40	41.29 ± 4.37*	69.82 ± 2.41
60	55.12 ± 2.27*	73.98 ± 2.35
80	59.21 ± 2.10*	79.94 ± 5.04
100	65.00 ± 4.37*	83.89 ± 4.10

Data are means ±SD of three measurement p< 0.05 compared to control*

CONCLUSION

The results of present study showed that methanolic extract of *Erythrina indica* have significant antioxidant activity, as tested through DPPH, reducing power, nitric oxide and amount of phenolic compound. The preliminary phytochemical investigation indicates the presence of flavonoids in the plant. Flavonoids are the well known natural antioxidants[19]. So, the antioxidant potential of the plant may be attributed to the presence of flavonoids.

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