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IN VITRO ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF ERYTHRINA VARIEGATA STEM BARK

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Abstract

Erythrina variegata leaves and bark are being used traditionally for the treatment of several diseases. In the present study, the methanol extract of Erythrina variegata stem bark has been assessed for the presence of phytochemicals and in vitro antioxidant activity by means of DPPH free radical and nitric oxide (NO) radical scavenging assays. Phytochemical analysis revealed the presence of flavonoids, glycosides, alkaloids, tannins, steroids, phenolics and diterpenes Methanol extract of Erythrina variegata stem bark exhibited marked scavenging activity against DPPH and NO free radicals in a dose dependent manner comparable to the effect produced by vitamin C standard. The EC_{50} value for DPPH free radical inhibition by the methanol extract of Erythrina variegata stem bark was 45.86±5. 85µg/mL whereas for nitric oxide radical inhibition, EC_{50} obtained was 28.14±3.42. Hence the present study identified that the methanol extract of Erythrina variegata stem bark possessed marked antioxidant activity in a dose dependent manner.

Key words : Antioxidant, DPPH, Erythrina variegata, Methanol extract, Nitric oxide.

I. INTRODUCTION

Medicinal plants are well known for their antioxidant activities. Secondary plant metabolites like phenolics and flavonoids are mainly concerned with antioxidant activity of plants. *Erythrina variegata* commonly known as 'Indian coral tree' belongs to the family fabaceae. It is found abundantly in tropical and subtropical regions of the Eastern Africa, Indian subcontinent and the islands of the Indian Ocean. It is a thorny deciduous tree which can grow upto 25 m height. *Erythrina variegata* is well known for antibacterial, antimalarial, antioxidant, antitumour and antidiabetic activities [1]. The present study aims at evaluation of antioxidant potential of methanol extract of stem bark of *Erythrina variegata*.

II. MATERIAL AND METHODS

2.1. Collection of plant material and authentication

Stem bark of *E. variegata* were collected from the premises of College of Veterinary and Animal Sciences, Mannuthy region of Thrissur district, Kerala. The plant material was taxonomically identified and authenticated by Dr. Anto. P. V. Assistant Professor, Research and Post Graduate Department of Botany, St. Thomas College, Thrissur, Kerala.

Voucher specimens of the plant has been deposited at the Herbarium of St. Thomas College (Collection No. 1) and at the Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur (HERB/VPT/CVASMTY/1/2017).

2.2. Methanol extraction

The stem bark of *Erythrina variegata* was washed, air dried at room temperature and coarsely powdered using an electric pulveriser. The powder obtained was extracted using a Soxhlet apparatus with methanol. The methanol extract was then concentrated using a rotary vacuum evaporator (Evator, Equitron EV11.ABI.029) under reduced pressure and temperature (55 ^{o}C) and kept under refrigeration after complete evaporation of the solvent in airtight container.

2.3. Qualitative phytochemical analysis

The methanol extract of bark of *Erythrina variegata* was tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, diterpenes, triterpenes and saponins [2].

2.4. DPPH free radical scavenging assay

1,1-Diphenyl-2- picryl- hydrazyl (DPPH) radical scavenging activity was measured using the method of [3] with some modifications. Three ml of the reaction mixture containing 2.5 ml of DPPH (100μM in methanol) and 0.5 ml of test solution at 3.9, 7.81, 15.625, 31.25, 62.5, 125, 250 and 500 µg/ml concentrations of extract were incubated at 37^{o} C for 10 min and absorbance of the resulting solution was measured at 517 nm using UV/VIS/NIR Spectrophotometer, Lambda 750, Perkin Elmer, Singapore. The reference standard taken for comparison was ascorbic acid. The per cent inhibition was calculated using the formula; Per cent inhibition of DPPH free radical = [(AC-AT)/AC] x 100, where, AC : Absorbance of the control and AT :Absorbance of the extracts/standard.

2.5. Nitric oxide scavenging assay

The nitric oxide scavenging activity of the extract was measured according to the modified method of [4]. To 2 ml of different concentrations of extract (3.9, 7.81, 15.62, 31.25, 62.5,125, 250 and 500 µg/ml), 0.5 ml of 5mM sodium nitroprusside solution in PBS (pH-7.4) was added and incubated for 2 hrs at room temperature. After incubation, added 1.2 ml of Griess reagent (equal volume of 1% sulfanilamide in 5% orthophosphoric acid and 0.1% naphthylethylene-diamine-dihydrochloride in distilled water) to the reaction mixture. The absorbance was read immediately at 546 nm against PBS blank and compared with vitamin C standard.

Nitric oxide scavenging activity (per cent) = [(AC-AT)/AC] x 100
where, AC : Absorbance of the control and AT : Absorbance of the extracts/standard.

The data of antioxidant assays are expressed as mean ±SE. The Effective Concentration 50 (EC_{50}) values of methanol extract of *Erythrina variegata* were calculated using the online software "Very Simple IC_{50} Tool Kit".

III. RESULTS AND DISCUSSION

Metabolic processes within the cell leads to generation of free radicals. Presence of an unpaired electron in the outer shell makes the free radical instable and highly reactive, which in excess leads to oxidative stress predisposing to mutagenic and carcinogenic changes in cell [5]. Hence, antioxidants play an important role in scavenging these free radicals and preventing the cells from toxic changes.

In the present study, the yield of the methanol extract of *E. variegata* bark was 21.78 per cent with reference to dry starting material. The methanol extract of *E. variegata* bark detected flavonoids, glycosides, alkaloids, tannins, steroids, phenolics and diterpenes upon phytochemical analysis (Table 1). The per cent inhibition of DPPH and NO generation by methanol extract of *E. variegata* at different concentrations are presented in Table 2. Methanol extract of *E. variegata*

exhibited good scavenging activity against DPPH and NO radical at the tested dose range of 3.9 to 500 µg/ml, in a dose dependent manner.

In DPPH free radical scavenging assay, antioxidant donates hydrogen atom which reduces DPPH to 1,1- diphenyl picryl hydrazine, a stable free radical indicated by purple to yellow colour change in a comparatively less time. In the present study, the extracts showed good reducing activity in a concentration dependent manner. The EC_{50} value for DPPH free radical inhibition by methanol extract of *E. variegata* was 45.86±5.85 µg/ml which were comparable to the EC_{50} value of 27.88 ±2.24 µg/ml, obtained for vitamin C standard. The results were in accordance with [6] in which *Tabebuia pallida* root bark extract showed EC_{50} of 48.5 ± 1.7 µg/ml when assessed using DPPH assay.

In nitric oxide radical scavenging assay, aqueous solution of sodium nitroprusside under physiological pH release nitric oxide which on reaction with oxygen releases nitrite radical, which is measured using griess reagent. *In vitro* nitric oxide radical scavenging activity of methanol extract of *E. variegata* stem bark was found to be higher than that of vitamin C at all the tested concentrations. EC_{50} value calculated for nitric oxide radical scavenging activity of methanol extract of *E. variegata* was 28.14 ±3.42 µg/ml while the EC_{50} noted for vitamin C standard was 76.27±3.93, which implies that compared to vitamin C standard, methanol extract of *E. variegata* stem bark is more potent in scavenging nitric oxide radical. . Nitric oxide free radical acts as effector for neuronal messenger, vasodilation, antimicrobial and antitumour activities. On chronic exposure, nitric oxide radical is associated with carcinomas, juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis. Interaction of nitric oxide with superoxide anion leads to generation of peroxynitrite anion which has a higher toxic potential. The results obtained were agreeable with [7] who obtained marked nitric oxide scavenging activity for chloroform soluble fraction of methanol extract of *S. glauca* with EC_{50} of 46.05 ± 4.09 µg/ml.

Phenolics and flavonoids, the polyphenolic compounds which primarily contribute to the free radical scavenging property of plant extracts [8] was also detected in methanol extract of *E. variegata* stem bark from our study. The results obtained by qualitative phytochemical analysis were in accordance with [9]. Thus the present study revealed that methanol extract of *Erythrina variegata* stem bark possessed marked antioxidant activity in a dose dependent manner. This study substantiates the popularity of *Erythrina variegata* in traditional medicine since free radical scavenging effect of phytochemicals can attribute to a multitude of pharmacological effects in biological systems.

IV. CONCLUSION

The results of the present study confirmed the *in vitro* antioxidant activity of the methanol extract of *Erythrina variegata* using DPPH free radical and nitric oxide free radical scavenging assays.

Table 1: Phytochemical analysis of methanol extract of E. variegata bark

Test	MEV
Steroids Salkowski's test	+
Alkaloids Dragendorff's test Mayer's test Wagner's test Hager's test	+ + + +
Glycosides Sodium hydroxide	+

test	
Tannins Ferric chloride test Gelatin test	 + +
Flavonoids Lead Acetate test Ferric chloride test	 + +
Diterpene detection test	+
Triterpenes Salkowski's test	 --
Saponins Foam test	 --
Phenolic compounds	+

Table 2. The percentage inhibition of DPPH and nitric oxide free radical generation by methanolic extract of E. variegata (MEV)

Concentrations (µg/mL)	% inhibition of Nitric oxide anion free radical		% inhibition of DPPH free radical	
	MEV	Vitamin C standard	MEV	Vitamin C standard
3.91	1.13±0.37	40.19±0.45	1.26±0.66	0.96±1.78
7.81	5.27 ± 1.21	41.21±0.41	1.76±0.79	5.54±2.24
15.62	11.56 ±1.19	42.18±0.17	2.59±1.04	25.18±2.39
31.25	17.69±0.19	45.63±0.11	6.96±0.27	49.37±2.61
62.5	24.09 ± 1.2	55.83±0.11	30.24±0.63	86.18±2.94
125	31.24±1.23	65.76±0.25	32.04±0.09	91.89±0.56
250	33.5 ± 1.91	71.83±0.69	35.77±0.13	93.92±0.21
500	34.98 ± 1.62	75.79±10.43	42.08±2.48	94.49±0.25
EC_{50}	**28.14±3.42**	**76.27±3.93**	**45.86 ± 5.85**	**27.88 ± 2.24**

Values are expressed as mean ± SE (n = 3)

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