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Phytochemical screening and "*in-vitro*" anti-oxidant activity of methanolic root extract of *Erythrina indica*

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ABSTRACT

Objective: *Erythrina indica* belongs to the family Leguminoseae and it is a medium-sized, spiny, deciduous tree normally growing up to 6-9 m tall. It is also known as "Indian coral tree" or "Tiger's clow" or "variegated coral tree" or "Kalyana murungai" or " Mulmurukku" (in Tamil). It is a native of costal forest communities from East Africa, through southeast to Australia. In India, it is distributed in coast forests from Bombay to Malabar . The objective of this study is to explore the phytochemistry and the antioxidant potential of methanolic root extract of Erythrina indica which is considered traditionally as an important medicinal plant. **Methods:** The preliminary phytochemical analysis was done to find out the presence of various bioactive compounds. In vitro antioxidant analysis of methanolic root extract was performed by 1,1diphenyl, 2 picryl hydrazyl assay, nitric oxide assay, superoxide dismutase assay, ferric reducing antioxidant power assay. **Results:** The methanolic root extract showed the presence of various phytoconstituents such as flavonoids, tannins, terpenoids, saponins, coumarins and carbohydrates. Besides it also possess strong antioxidant activity. **Conclusions:** It was concluded that *Erythrina indica* root possessed a wide range of pharmacologically important phytoconstituents which exhibited strong antioxidant activity.

1. Introduction

Free radicals or ROS are formed in our body as result of biological oxidation; Over production of the same contributes to the oxidative stress, [1,2] which leads to the damage of proteins, DNA and lipid that is associated with the chronic degenerative diseases including cancer , coronary artery disease, hypertension and diabetes etc. [3]

Most ROS are scavenged by endogenous defense systems such as catalase, superoxide dismutase, peroxidase-glutathione system etc. [4] But these systems may not be completely efficient, making them to depend on exogenous anti-oxidants from natural sources. The recent abundant evidences suggest that reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide and hydroxyl radical, involve in the pathogenesis of various disorders and diseases [5].

The safety of synthetic antioxidants in food industry has become a concern among scientist and leading current interest in uncovering natural antioxidants [6]. According to the WHO "A medicinal plant is a plant which, in one or more of its organs, contains bioactive substances that can be used for therapeutic purposes or which are precursors for chemo-pharmaceutical semi-synthesis"[7].

The role of medicinal plants in disease prevention or control has been attributed to the anti-oxidant properties of their constituents such as vitamins, terpenoids, phenolicacids, stilbenes,tannins,flavonoids,quinones,coumarins,alkaloids, which are rich in anti-oxidant activity[8]. The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing. [9,10]

The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds scavenge free radicals and thus inhibit the oxidative mechanisms that lead to degenerative diseases [11]. As in the case in the other countries of the world, in recent years, the plants used traditionally for curative purposes, have attracted the attention of researchers. [12-15]

2. Materials and methods

2.1 Collection and authentication

For this study roots of *Erythrina indica* were collected from Vazhappadi town, near Salem district, Tamilnadu

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state. The collected roots were botanically authenticated by Prof. K. Murugesan, Botanist, University of Madras and a voucher specimen was also deposited for future reference. The roots were chopped and ground to a coarse powder.

2.2. Chemicals

TPTZ(2,4,6-tri(2-pyridyl)-s-triazine), dilute Hcl, ferric chloride, ferrous sulphate, potassium ferricyanide, trichloro acetic acid, sodium nitrite, nitro blue tetrazolium (NBT), nicotinamide adenine dinucleotide (NAD), phenazine methosulfate(PMS), DPPH, ascorbic acid, aluminium chloride, potassium acetate were purchased from Hi Media. Solvents used for the extraction and other chemicals used were of analytical grade unless otherwise indicated.

2.3. Preparation of extract

Approximately two kilogram of the roots were collected and washed in running tap water. The roots were then chopped in to small pieces and shade dried for about a month and finely ground to coarse powder in a blender. The powder was then extracted with methanol using Soxhlet apparatus and concentrated in rotary evaporator under reduced pressure and dried. The dried powder was diluted with saline and used for the study.

2.4. Estimation of total flavonoids

The total soluble flavonoid content was estimated by aluminium chloride colorimetric method [16] for methanolic extract 0.5ml of stock solution in (1g/ml) of the extract, 1.5 ml methanol, 0.1ml potassium acetate (1M) was added to reaction test tubes and volume was made up to 5 ml with distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by using quercetin as the standard at concentrations of 20 -100 μg in methanol.

2.5. Estimation of total phenols

Total soluble phenolic content was estimated by Folin-Ciocalteau reagent method [17] using gallic acid as a standard. One ml of stock solution of methanol extract was prepared (1g/ml) from which different aliquots were pipetted out into test tubes. The volume was made up to 3 ml with distilled water to which freshly prepared Folin-Ciocalteau reagent was added. After 3 min, 2 ml of 20% sodium carbonate solution was added to each tube and mixed thoroughly. The tubes were placed in boiling water for one minute, cooled and the absorbance was measured at 650 nm in a spectrophotometer against a reagent blank. The concentrations of the total phenolic compounds in the extracts were obtained by extrapolating the absorbance of gallic acid on standard gallic acid graph. The experiment was repeated thrice and concentration of total phenols was expressed as μg/mg of dry extract.

2.6. In vitro antioxidant assay

2.6.1. Frap assay

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay of Benzie *et al* [18] FRAP assays uses antioxidants as reductant in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. 10 μl of sample (1 mg/ml) was made up to 1 ml with distilled water and was mixed with 1.5ml of working FRAP reagent and incubated at 37℃ for 4 minutes. After incubation the absorbance was measured at 593nm. Ferrous sulphate standard was processed in the same way and the FRAP value was calculated from the standard graph.

2.6.2. Dpph assay

Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPHH and as consequence the absorbance′s decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

The DPPH free radical scavenging activity of each sample was determined using the UV/vis Spectrophotometer according to the method described by Leong *et al* [19] Briefly, a 0.1mM solution of DPPH in methanol was prepared. The initial absorbance of the DPPH in methanol was measured at 515 nm and did not change throughout the period of assay. An aliquot (40 μl) of an extract (with appropriate dilution, if necessary) was added to 3 ml of methanol DPPH solution. The change in absorbance at 515 nm was measured at 30 min of incubation.

The free radical-scavenging activity (FRSA) was calculated using the formula as follows,

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

2.6.3. Nitric oxide scavenging assay

Nitric oxide was generated from nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide [20,21] which interacts with oxygen to produce nitric oxide which, interacts with oxygen to produce nitric ions that can be estimated by use of Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide [22]. Sodium nitroprusside (5 mM) in phosphate- buffered saline (PBS) was mixed with 3.0 ml of different concentrations of the drugs dissolved in the methanol and incubated at 25℃ for 150 min. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm. The experiment was done in triplets.

Standard Sodium nitrite (10mg/100ml) standard was processed in the same way and the inhibition percentage was calculated.

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

2.6.4. Superoxide radical scavenging assay

The measurement of superoxide anion scavenging activity was based on the method by Fontana, *et al* [23]. Superoxide radical is generated in phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT) to a purple formazan. The 1 ml reaction mixture contained phosphate buffer (20 mM, pH 7.4), NADH (73 μM), NBT (50 μM), PMS (15 μM) and various concentrations of sample solution. After incubation for 5 min at ambient temperature, the absorbance at 562 nm was measured against an appropriate blank to determine the quantity of formazan generated. The experiment was repeated thrice.

The results were compared with that of standard quercetin. The % inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

Figure 1. *Erythrina indica* tree. Location of tree : Vazhappadi town, Salem District, Tamil Nadu state.

3. Results

3.1.Phytochemical analysis

The methanolic root extract of E.indica was screened for the presence of various bioactive phytochemical compounds. Specific qualitative tests were performed to identify bioactive compounds of pharmacological importance through standard methods. The analysis revealed the presence of carbohydrates and quinones, in most prominent amount while phenols, flavonoids, steroids, coumarins and terpenoids and tannins in less amount. These results were documented in Table 1.

Table 1.
Phytochemical analysis of methanolic root extract of E.indica:

Phytochemical test	Methanol extract	
	Observation	Inference
Carbohydrates	Red color	++
Tannins test	Greenish black color	+
Saponin test	Presence of foam	+
Flavonoid test	Yellow color	+
Quinones	Red color	++
Terpenoids test	Red brown color	+
Phenols	color change	+
Coumarins	Yellow color	+
Steroids and Phytosteroids	color change	+

+ Present; ++ Strongly Present; − Absent

3.2. Estimation of total flavonoids

Total flavonoid content in the roots of *Erythrina indica* was 42.5 μg/mg. The result indicates strong association between antioxidative activities and flavonoid compounds (r2= 0.9972), (Fig.2) suggesting that Flavonoid compounds are probably responsible for the antioxidative activities of *Erythrina indica*.

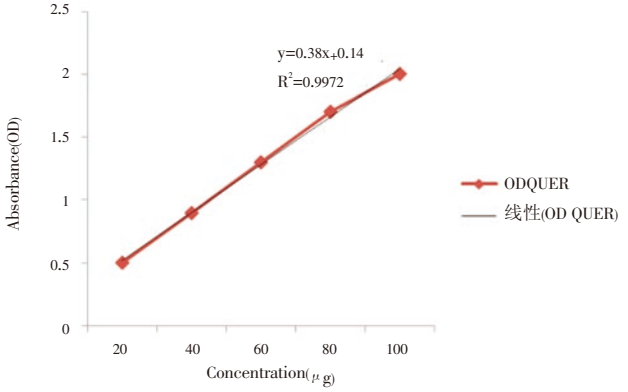

Figure 2. Standard curve for determination of quercitin equivalents for total flavonoid assay.
Quer: Quercetin, OD: Optical Density.

3.3. Estimation of total phenolic content

In *Erythrina indica* 55 μg/mg Gallic acid equivalent of phenol was detected. The results indicated that there was a strong association existed between antioxidative activities and phenolic compounds (r2 = 0.9985), suggesting that phenolic compounds are probably responsible for the antioxidative activities of *Erythrina indica*. Studies have reported a high correlation between phenolic content and antioxidant activity [24]. Phenolic compounds may be the effective hydrogen donors, making them good antioxidants (table 3) [25].

3.4. Antioxidant activity of methanolic root extract of E.indica

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. The E. indica root extract demonstrated H-donor activity. The DPPH radical scavenging activity was detected and compared with ascorbic acid. Figure 4 showed the percentage inhibition values of Ascorbic acid and *Erythrina indica* were 55.67% and 61.15 % respectively.

Erythrina indica effectively reduced the generation of nitric oxide from sodium nitroprusside. Fig.4 showed that percent inhibition values of E.indica and sodium nitrite standard were 25.78%, 46.87% respectively.

Fig.4 showed the superoxide inhibition values of *Erythrina indica* and standard were 35.74%, 64.24% respectively. Results obtained emphasized the capacity of *Erythrina indica* extracts to annihilate the superoxide anions generated through PMS-NADPH-NBT system.

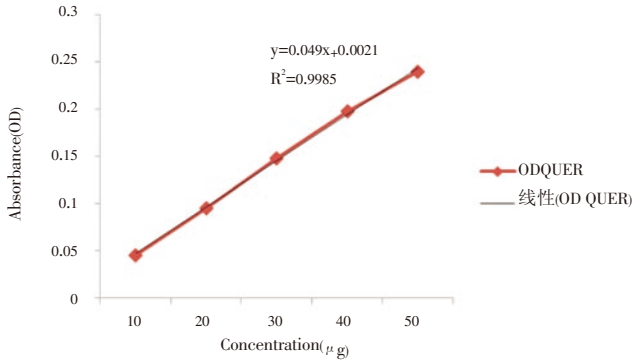

Figure 3. Standard curve for determination of gallic acid equivalents for total phenol assay.
Gae: Gallic acid.OD: Optical Density.

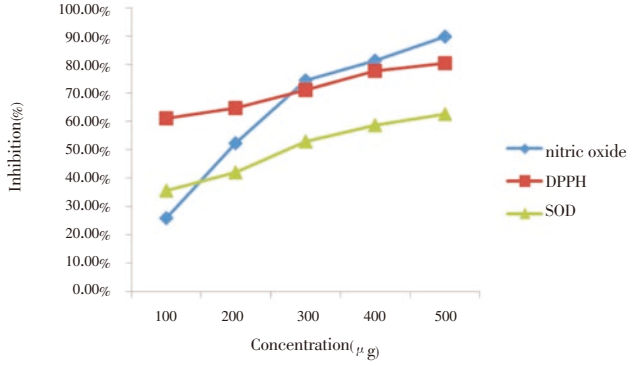

Figure 4. Comparative graph of Nitric oxide scavenging assay, DPPH assay and Superoxide dismutase assay of methanolic root extract of E.indica. Nitric oxide assay shows maximum inhibition of 89.84% in 500 μg concentration with comparison to DPPH and SOD which has 80.24% and 62.45% inhibition respectively.
Dpph: 2,2 Diphenyl picryl hydrazyl. SOD: Superoxide dismutase.

3.5.Frap assay

The ability of the plants extracts to reduce ferric ions was determined using the FRAPassay . An antioxidant capable of donating a single electron to the ferric-TPTZ (Fe(III)-TPTZ) complex would cause the reduction of this complex into the blue ferrous-TPTZ (Fe(II)-TPTZ) complex which absorbs strongly at 593 nm.

Figure 5: Frap assay:The FRAP values for the extracts were greater than that of ferrous sulphate standard with 7500 μM/mg FRAP value at 40 μL for methanolic root extract showing potent ferric reducing power of *Erythrina indica* root. (Fig 5, Table 2).

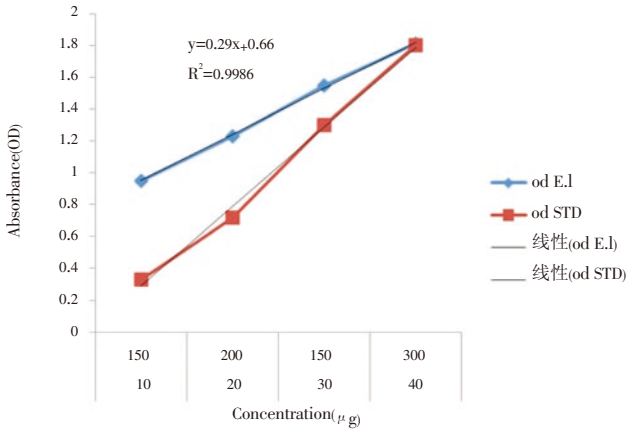

Figure 5. Comparitive linear graph of *E.indica* and standard ferrous sulphate.
FRAP: Ferric reducing antioxidant power assay, E.i: *Erythrina indica*, Std: Standard.

Table 2.
Frap assay of methanolic root extract of Erythrina indica

Concentration (μL)	Absorbance	Frap value(μM/mg)
10	0.330	5800
20	0.720	6000
30	1.300	7033.33
40	1.800	7500

4. Discussion

The high DPPH activity could be correlated with high phenolic content. Literature survey revealed high level of phenolic content showed fast decrease in absorbance of DPPH radical [26]. DPPH is a stable, nitrogen-centered free radical which produces violet colour in methanol solution. It was reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the methanolic root extract in a concentration-dependent manner.

Scavenging of nitric oxide radical is based on the generation of nitric oxide. Sodium nitroprusside in buffered saline, reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent. *Erythrina indica* decreased the amount of nitrite generated from the decomposition of sodium nitroprusside in vitro.

Superoxide anions are a precursor to active free radicals, which is normally formed first in cellular oxidation reactions. Although, it is not highly reactive, it can produce other ROS such as hydrogen peroxide, hydroxyl radical, and singlet oxygen. Furthermore, superoxide anion radical and its derivatives can cause damage in lipids, proteins, and DNA. Therefore, it is of great important to scavenge superoxide anion radical [27].The methanolic root extract of E.indica exhibited significant superoxide dismutase activity (Fig-4). The literature survey showed that the FRAP assay is sensitive method for the measurement of total antioxidant power of the fresh biological fluids such as plant

homogenates and pharmacological plant products [28]. The methanolic root extract of E.indica exhibited high FRAP value even at the very lower concentration.(Table 2)

The findings of the present study suggest that *Erythrina indica* could be a potential source of natural antioxidant that could have great importance as therapeutic agent in preventing or slowing the oxidative stress related degenerative diseases. Further work regarding isolation of bioactive compounds responsible for this potent antioxidant activity will be carried out.

Conflict of interest statement

We declare that we have no conflict of interest.

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