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Evaluation of Antioxidant and DNA Nicking Potential of Anthocyanin Extracted from *Erythrina indica* Flower Petals.

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ABSTRACT

The *Erythrina indica* is a widely distributed plant. In the present study, *Erythrina indica* was used as a source for anthocyanin extraction. Anthocyanins are natural pigments, which are responsible for red, purple and blue colours in parts of the plant. It belongs to a major flavonoid class which has strong antioxidant activity. In the present scenario, there is a rising demand for natural sources of food colorants with nutraceutical benefits. So the present research focus on the antioxidant analysis of anthocyanin extracted from *Erythrina indica* flower petals. Antioxidant assays were performed by DPPH, Superoxide radical and FRAP activity. From the results, it was confirmed that anthocyanin extracted from *Erythrina indica* has potent antioxidant properties. Finally, ensure that the anthocyanin extracted replace many synthetic colourant and chemical antioxidants.

Keywords: *Erythrina indica,* anthocyanin, antioxidant, DNA nicking

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INTRODUCTION

Antioxidants are substances known to protect the cells against the effect of free radicals through scavenging free radicals and singlet oxygen. But lipids, proteins, nucleic acids and carbohydrates are all known to undergo oxidative modification. Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, ageing process and perhaps mentias. Elimination of synthetic antioxidants in food applications has given more impetus to exploring natural sources of antioxidants [1]. Plants synthesize a vast range of secondary metabolites with a significant portion consisting of phenolic compounds and flavonoid compounds [2]. Flavonoids, anthocyanins and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties.

Erythrina indica is a medium-sized, spiny, deciduous tree normally growing to 6-9 m (occasionally 28 m) tall and 60 cm. *Erythrina* comes from the Greek word 'eruthros' meaning red, alluding to the showy red flowers of the *Erythrina* species. Young stems and branches are thickly armed with stout conical spines up to 8 mm long, which fall off after 2-4 years; rarely, a few spines persist and are retained with the corky bark. Bark smooth and green when young, exfoliating in papery flakes, becoming thick, corky and deeply fissured with age. Leaves trifoliate, alternate, bright emerald-green, on long petioles 6-15 cm, rachis 5-30 cm long, prickly; leaflets smooth, shiny, broader than long, 8-20 by 5-15 cm, ovate to acuminate with an obtusely pointed end. Leaf petiole and rachis are spiny. Flowers are bright pink to scarlet erect terminal racemes 15-20 cm long; stamens slightly protruding from the flower. Fruit are cylindrical torulose pod, green, turning black and wrinkly as they ripen, thin-walled and constricted around the seeds. There are 1-8 smooth, oblong, dark red to almost black seeds per pod. *Erythrina indica* has several medicinal property, the bark decoction is used in stomach disorders, anti-abortion treatment, malarial fever and liver problems. The present study was undertaken to evaluate the anthocyanin content of *Erythrina indica* and to examine its antioxidant activity.

MATERIALS AND METHODS

Sample collection

Erythrina indica were collected from in and out of kodaikanal hills, Dindigul district, Tamil Nadu

Extraction

0.5gm of *Erythrina indica* flower petals were treated with 10 ml acidified methanol. And the mixture was centrifuged at 10,000 rpm for 10 min and supernatant was taken for analysis [3].

Analytical procedures [4]

Flavanoids conformation test

$Fecl_3$

1ml of sample extraction was added with a small amount of $FeCl_3$**,** and results was observed.

$Alcl_3$

1ml of sample extraction was added with 5% of $AlCl_3$ solution, and results was observed.

Conformation test for anthocyanin

2M HCl

1ml of sample extraction was added with 2ml of HCl and keep for 5minutes at 100ºC, and the result was observed.

2M NaoH

1ml of sample extraction was added with 2ml of NaoH, and results was observed.

Total phenolic assay

Total phenolic compounds in anthocyanin samples were quantified by using Folin ciocalteu's method described by Ronald [5]. 1ml of Folin-ciocalteu's reagent (50% v/v) were added to 100µl of sample extract. It was incubated for 5 min. After incubation 1ml of 20 % (w/v) sodium carbonate and water was added to final volume of 4ml. Blank was prepared by replacing the reagent by water to correct for interfering compounds. After 30 min of incubation, the absorbance was measured using spectrophotometer at 760 nm.

Determination of total anthocyanin

The total amount of anthocyanin content was determined by using pH differential method. A spectrophotometer was used for the spectral measurements at 210 nm and 750nm [6]. The absorbance of the samples (A) was calculated as follows:

A= (Absorbance λ vis-max-A750) pH 1.0-(Absorbance λ vis-max-A750) pH 4.5
Anthocyanin pigment content (mg/litre) = (A X MW X DF X 1000)/ (ε X 1).

Where,

Molecular weight of anthocyanin (cyd-3-glu) = 449, Extraction coefficient (ε) = 29,600, DF =Diluted factor.

Stability at variable pH

The anthocyanin stability was tested by treating 1ml of sample with 1 ml of pH 1.0 and 4.5 solutions. The color change was observed [7].

Antioxidant assays

Scavenging activity of DPPH radical

Scavenging activity of Anthocyanins against DPPH radicals was assessed according to the method of [8] with some modifications. Briefly, 0.1 mM DPPH-methanol solution was mixed with 1 ml of 0.1mM DPPH methanol solution. After the solution was incubated for 30 min at 25º C in dark, the decrease in the absorbance at 517nm was measured. Control contained methanol instead of antioxidant solution while blanks contained methanol instead of DPPH solution in the experiment. Ascorbic acid and BHT were used as positive controls. The inhibition of DPPH radicals by the samples was calculated according to the following equation:

DPPH-scavenging activity (%) = [1-(absorbance of the sample-absorbance of blank)/absorbance of the control] *100

Determination of superoxide radical-Scavenging activity

Superoxide radicals were generated by the method of Ginnopolites and Ries (1977), described by [9], with some modifications all solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The photo induced reactions were performed in aluminium foil-lined box with two 30W fluorescent lamps. The distance between the reaction solution and the lamp was adjusted until the intensity of illumination reached about 4000 lux. A 30µL aliquot of *Erythrina indica flower* anthocyanin mixed with 3ml of reaction buffer solution (1.3 mm riboflavin, 13 mM methionine, 63 µM nitro blue tetrazolium and 100µM EDTA, pH 7.8). The reaction solution was illuminated for 15 min at 25 º C. The reaction mixture, without sample, was used as a control. The scavenging activity was calculated as follows:

Scavenging activity (%) = (1-absorbance of the sample/absorbance of control) ×100

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed according to [10, 11] with some modifications. The FRAP assay reagent was prepared by adding 10 volume of 300mM acetate buffer, pH 3.6 (3.1g sodium acetate and 16ml glacial acetic acid), one volume of 10mM TPTZ prepared in 40mM HCL and one volume of 20 mM $FeCl_3$. The mixture was diluted to 1/3 with methanol and prewarmed at 37ºC. This reagent (3ml) was mixed with 0.1 ml diluted test samples. The mixture was shaken and incubated at 37ºC for 8 minutes and the absorbance was read at 593nm. A blank with only 0.1ml methanol was used or calibration. FRAP assay was expressed as inhibition percentage and was calculated using the formula**:**

FRAP assay % = 1-(test sample absorbance/blank sample absorbance) ×100.

DNA nicking assay

DNA nicking assay was performed using genomic DNA by the method [12]. A mixture of 5µl of *Erythrina indica* anthocyanins extract and genomic DNA was incubated for 10min at room temperature followed by the addition of 10µl of Fenton's reagent (30mM H_20_2, 50 µM ascorbic acid and 80 µM FeCl3). The final volume of the mixture was made up to 20 µl and incubated for 30 min at 37ºC. The DNA was analysed on 1 % agrose gel using ethidium bromide staining.

RESULTS AND DISCUSSION

Preliminary screening

The sample extracted with acidified methanol as solvent was tested for the presence of anthocyanin. In the presence of $FeCl_3$ the acidified extracts showed brown color in $AlCl_3$ dark color was observed which conforms the presence of flavanoids. The extract treated with 2M HCl was found to be stable and when treated with 2M NaoH it showed green color which confirms the presence of anthocyanin [4]. The UV spectra revealed the presence of anthocyanin pigments with absorption maxima at 400 to 700nm (Figure.1) which conforms it is typical a pigment [4, 13].

Stability of anthocyanin

No colour change was observed at pH 1 and pH 4.5, Previous report denote that [14], the anthocyanins are stable in low pH. The results were found to be same in the extracts of *Erythrina indica*.

Total anthocyanin and phenol content

The total anthocyanin content in the acidified methanol extracts of *Erythrina indica* flower petals was found to be 13.94mg/litre and the total phenolic content was determined as 290mg/g when compared with standard Gallic acid. Flowers, fruits and vegetables have health benefits and are good sources of phenolics, flavonoids, anthocyanins and carotenoids [15]. However, we found considerable amount of phenol and anthocyanins content in the *Erythrina indica* flower petals.

Antioxidant assays

DPPH

The ability of phenolic compound to quench reactive species by hydrogen donation was measured through the DPPH radical scavenging activity assay. Activity is measured as the

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reactive decrease in absorbance at 517nm as the reaction between DPPH and antioxidant progresses [16, 17]. The anthocyanin extract of *Erythrina indica* exhibited DPPH activity of 63.08% (table 1). In previous study reports that the anthocyanins significantly inhibited the activity of DPPH radicals in a dose-dependent manner in litchi chinenesis which shows anthocyanin have a strong hydrogen-donating capacity and can efficiently scavenge DPPH radical [18].

Figure 1: UV-Vis sepectral analysis of *Erythinia indica* flower

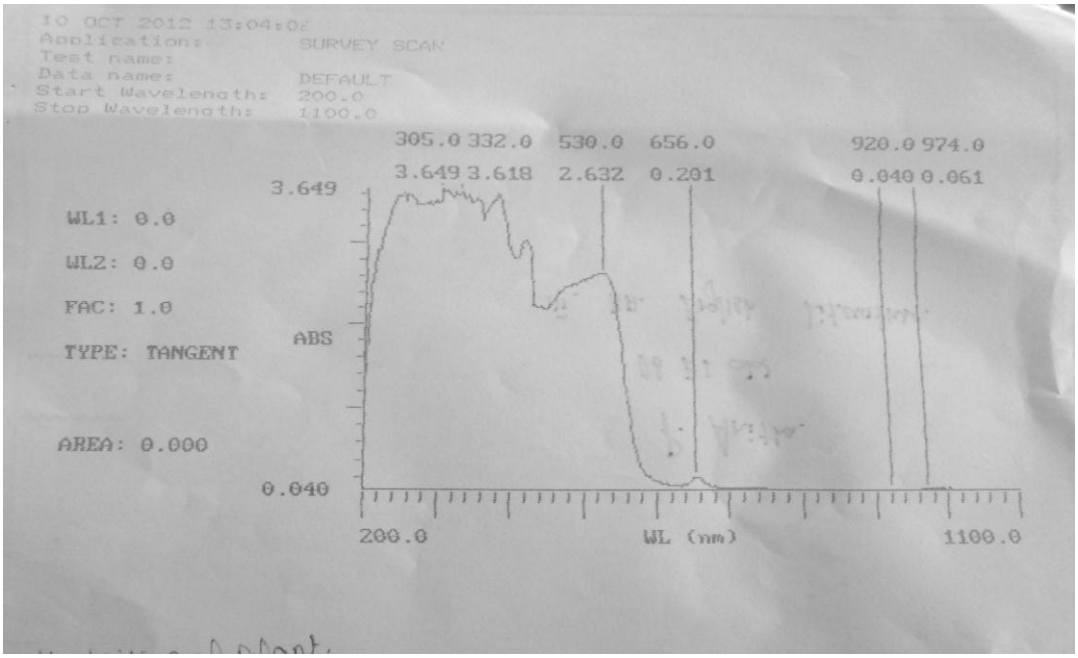

Figure 2: Antioxidant analysis of anthocyanin from *Erythrina indica* in FRAP assay

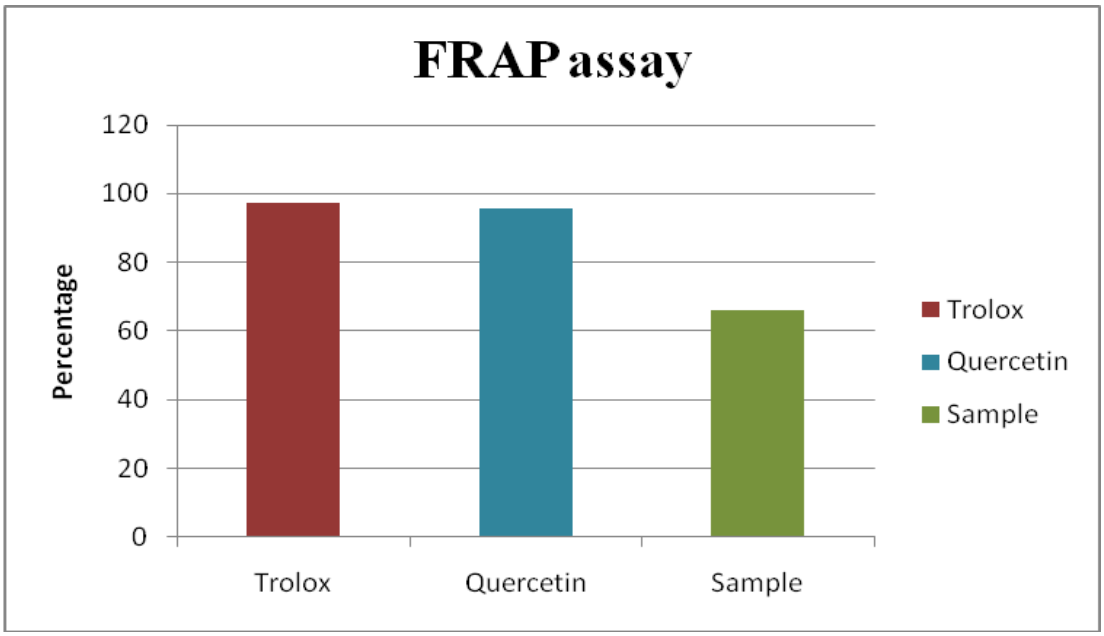

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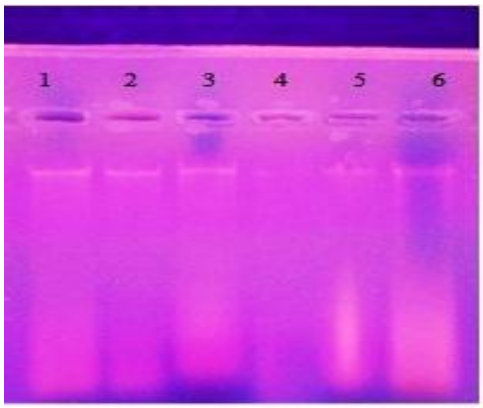
Table 1: Antioxidant assays of anthocyanin extracted from *Erythrina indica*

Antioxidant assays	Standards		Sample (*Erythrina indica)*
	BHT	Ascorbic acid	
DPPH assay	95.8	96.6	63.8%
Superoxide radical activity	61	69.5	28.2%

Figure 3: DNA nicking assay of anthocyanin extracted from *Erythrina indica*



Lane 1: DNA + Fenton's reagent + 150µg sample + water
Lane 2: DNA + Fenton's reagent + 300µg sample + water
Lane 3: DNA
Lane 4: DNA + Fenton's reagent
Lane 5: DNA + Fenton's reagent + Methanol
Lane 6: DNA + Fenton's reagent + Quercetin

Superoxide radical scavenging

Superoxide radical can be generated by illuminating a solution containing riboflavin. Further superoxides are also known to indirectly initiate lipid peroxidation as a result of H_2O_2 formation, creating precursor of hydroxyl radicals [19]. The anthocyanin extract of *Erythrina indica* exhibited superoxide scavenging activity of 28.2% (Table.1). The superoxide anion on radical scavenging acitivity might be due to the action of phenolic compounds. Also the flavanoid molecules with polyhydroxylated subsititution on the rings A and B and a free three hydroxyl substitution confirmed superoxide anion scavenging acitivity [9].

FRAP

FRAP assay is presented as a novel method of assessing total antioxidant capacity and is considered as a useful indicator of the body's antioxidant status to counteract the oxidative damage due to ROS. The advantage of the FRAP assay is in being fast, easy to handle, with highly reproducible results. The *Erythrina indica* flower petal anthocyanin extract showed 66% of activity when compared with standards (Trolox and Quercetin) that shows the antioxidant activity which prevents the oxidative damage (Figure.2). The antioxidant activities of purple potato illustrated by DPPH and FRAP assays showed high correlation with anthocyanins [20].

DNA nicking

Hydroxyl radicals generated by the Fenton reaction are known to cause oxidatively induced breaks in DNA strands to yield its fragmented forms. The free radical scavenging effects of Erythrina indica anthocyanin were studied on Genomic DNA damage. The anthocyanins showed significant reduction in the formation of nicked DNA and increased native form of DNA (Figure.3). Quercetin effectively protected DNA strand scission from tert-butyl hydroperoxide [21]. The aqueous extract of *Curcuma amada* was found to protect against Herring sperm DNA [22].

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