



## Phytochemical Analysis, Cytotoxic and *In vitro* Antioxidant Activity of *Erythrina variegata* Bark

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors MS, NZK and NA designed the study. Authors MK, MSIB and SJC managed the analyses of the study. Authors SNN and RA performed statistical analysis. Authors MS and RA wrote the protocol. Authors MS, NZK and NA wrote the first draft of the manuscript. Authors ZS, SNN and SFC contributed the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

**Aim:** The objective of the present study was to investigate the phytochemical analysis, cytotoxic and antioxidant activities of the bark extracts of *Erythrina variegata*.

**Place and Duration of Study:** The plant, *E. variegata* was collected from Botanical Garden, Mirpur, Dhaka and also from Curzon Hall, University of Dhaka, Bangladesh in June 2014 and then the sample was identified by the National Herbarium of Bangladesh, Mirpur, Dhaka (DACB; Accession Number- 36148).

**Methodology:** Preliminary phytochemical screening was done for determining the nature of phytoconstituents. Brine shrimp lethality bioassay technique was done to study the cytotoxic

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properties. Aluminum chloride colorimetric method was applied for the determination of flavonoids and the total antioxidant ability was assessed by the phospho-molybdenum method.

**Results:** Primary phytochemical analysis confirmed the presence of alkaloid, carbohydrate, glycosides and flavonoids. The extracts showed some toxicity to *A. salina* with LC<sub>50</sub> values ranging from 1.41 to 3.66 µg/ml which was compared with standard vincristine sulphate (VS, LC<sub>50</sub> value 0.92 µg/ml). n-Hexane extract of *E. variegata* was found to contain highest amount of flavonoids (3.77±1.97 mg/gm; quercetin equivalent) and ethanol extract of *E. variegata* was found to have highest antioxidant activity (2.03±0.09 mg/gm ascorbic acid equivalent).

**Conclusion:** It can be concluded that the plant extracts of *E. variegata* possess several antioxidant activities which justifies its use as folk medicine.

*Keywords:* *Erythrina variegata*; phytochemicals; antioxidant capacities; cytotoxicity.

## 1. INTRODUCTION

Traditionally medicinal plants are widely used for the treatment of many diseases. The plants may contain chemical compounds which have the property of drugs or can act as precursor for the synthesis of useful drugs [1]. The ancient literatures include many plants for their medicinal values and they have been found to be effective for the treatment of various diseases which play significant role in providing primary healthcare services to rural and poor people who cannot afford costly and rare synthetic drugs. Moreover, these plants can act as a major source of income by exporting medicinal plants to other countries [2].

*Erythrina variegata* belongs to Fabaceae family. The genus, *Erythrina* includes about 110 species which are collectively known as "coral tree". The coral tree is cultivated particularly as an decorative tree and as a shade and also as a soil improvement tree (it fixes nitrogen) for other trees and crops such as coffee and cacao [3]. The plant parts from *E. variegata* are widely used for the treatment of different disease complications. The leaves are employed for their antispasmodic, anthelmintic, laxative, diuretic, galactagogue and emmenagogue properties. They are also applied externally for dispersing venereal buboes, relieving pain and inflammation of the joints [4]. Its juice is used to the ear to relieve ear ache and is used as an anodyne in toothache. The bark is used for its astringent, febrifuge anti-bilious and anthelmintic properties; It is also useful in dysentery and as a collyrium for ophthalmia. The roots are emmenagogue [5].

The objective of the present study was to investigate the phytochemical properties, *in vitro* cytotoxic activity and *in vitro* antioxidant activities of the bark extracts of *E. variegata*.

## 2. MATERIALS AND METHODS

### 2.1 Collection, Identification and Processing of Plant Sample

The bark extracts of *Erythrina variegata* was collected from the Botanical garden, Dhaka and Curzon Hall, University of Dhaka in June 2014 and was taxonomically identified with the help of the National Herbarium of Bangladesh, Mirpur-1, Dhaka (DACB; Accession Number- 36148). Bark was sun dried for seven days. The dried bark were then ground in to coarse powder using high capacity mixer grinding machine (Jaipan designer mixer grinder, jaipan, India), which was then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

### 2.2 Extraction Procedure

The powdered plant parts (500 gm) were successively extracted in a soxhlet extractor at elevated temperature using 250 ml of distilled methanol (40-60)°C which was followed by ethanol, chloroform, petroleum and n-hexane. After extraction all extracts were labeled and kept in refrigerator at 4°C for future investigation.

### 2.3 Preliminary Phytochemical Screening

One gram of the methanol extract of *Erythrina variegata* was dissolved in 100 ml of methanol and was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents [5,6].

### 2.4 Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay [7,8] technique was applied for the determination of general toxic properties of the plant extracts. Dimethylsulfoxide (DMSO) solutions of the samples were applied against *Artemia salina* in a 1-day *in vivo* assay.

For the experiment, 1 mg of each extracts were added with 5 ml of sea water. Concentration was found to be 200 µg/ml. Then 50 µl DMSO was added to these and sample was prepared. Then the solution was serial diluted to 100, 50, 25, 12.5, 6.25, 3.125, 1.563 µg/ml with sea water. Then 2.5 ml of plant extract solution was added to 2.5 ml seawater containing 10 nauplii. Vincristine Sulphate (VS) was used as positive control.

## 2.5 Determination of Total Flavonoids Content

Aluminum chloride colorimetric method was used for flavonoids determination [9]. 1 ml of the plant extracts/standard of different concentration solution was mixed with 3 ml of methanol, 0.2 ml of aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. It was then kept at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm wavelength with UV-VIS spectrophotometer (UV-1280, UV-VIS spectrophotometer, Shimadzu, Japan) against blank. Methanol was used as blank. The total content of flavonoid compounds in methanol extracts of the plant in quercetin equivalents was calculated by the following equation:

$$C = (c \times V) / m$$

where; C = total content of flavonoid compounds, mg/gm plant extract, in quercetin equivalent, c = the concentration of quercetin established from the calibration curve in mg/ml, V = the volume of extract in ml and m = the weight of crude plant extract in gm.

## 2.6 Determination of Total Antioxidant Capacity

The total antioxidant capacity was evaluated by the phosphomolybdenum method [10,11]. 0.3 ml of extract and sub-fraction in ethanol, ascorbic

acid used as standard (5-200 µg/ml) and blank (ethanol) were combined with 3 ml of reagent mixture separately and incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance of each sample was measured at 695 nm against the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid and was calculated by the following equation:

$$A = (c \times V) / m$$

where, A = total content of Antioxidant compounds, mg/gm plant extract, in Ascorbic acid Equivalent, c = the concentration of ascorbic acid established from the calibration curve, mg/ml, V = the volume of extract in ml, m = the weight of crude plant extract, gm.

## 3. RESULTS AND DISCUSSION

### 3.1 Preliminary Phytochemical Screening

In primary phytochemical screening, methanol, ethanol, pet-ether and chloroform extracts of *E. variegata* was found to contain alkaloid, carbohydrate, glycosides and flavonoids although the saponin was absent in chloroform, pet-ether and n-hexane extracts. Phenols, steroids and tannins were absent in all the extracts (Table 1).

### 3.2 Brine Shrimp Lethality Bioassay

In the brine shrimp lethality bioassay the LC<sub>50</sub> value of the test samples after 24 hours was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration. The best-fit line was obtained from the curve data by means of regression analysis.

Vincristine Sulphate (VS) was used as positive control, LC<sub>50</sub> was found to be 0.92 µg/ml.

**Table 1. Analysis of phytochemicals in the different extracts of *Erythrina variegata***

Name of tests	Name of extracts				
	Methanol	Ethanol	Chloroform	Pet-ether	n-Hexane
Alkaloids	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Flavonoids	+	+	+	+	+
Glycosides	+	+	+	+	+
Phenols	-	-	-	-	-
Saponins	+	+	-	-	-
Steroids	-	-	-	-	-
Tannins	-	-	-	-	-

(+) Presence & (-) Absent

The LC<sub>50</sub> values of methanol, ethanol, chloroform, n-hexane and pet-ether extracts of *E. variegata* are presented in Table 2.

**Table 2. LC<sub>50</sub> values of the five extracts of *Erythrina variegata* and standard**

Test samples	LC <sub>50</sub>
Vincristine sulphate (standard)	0.92
Methanol	3.66
Ethanol	3.12
Chloroform	1.45
n-Hexane	1.41
Petroleum ether	1.92

### 3.3 Total Flavonoid Content

Aluminium chloride colorimetric method was used to determine total flavonoid content of different extracts of *E. variegata*. Total flavonoids content was determined using the standard curve of quercetin ( $y = 0.690x - 0.689$ ;  $R^2 = 0.761$ ) and was expressed as Quercetin Equivalents (QE) per gram of the plant extract. N-Hexane extract of *E. variegata* was found to contain the highest amount of flavonoids (Table 3).

**Table 3. Total flavonoid contents of the different extracts of *Erythrina variegata***

Name of extracts	Total flavonoid content (mg/gm; quercetin equivalent)
Methanol	2.09±0.07
Ethanol	2.26±0.11
Chloroform	2.33±0.32
Petroleum ether	3.55±1.63
n-Hexane	3.77±1.97

*Values are the mean of duplicate experiments and represented as mean±SD*

### 3.4 Total Antioxidant Capacity

Total antioxidant capacity of the different extracts of *E. variegata* was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents per gram of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid ( $y = 1.076x - 0.808$ ;  $R^2 = 0.953$ ). The ethanol extract of was found to possess highest amount of antioxidant (Table 4).

**Table 4. Total antioxidant capacity of the different extracts of *Erythrina variegata***

Name of extracts	Total anti-oxidant capacity (mg/gm), ascorbic acid equivalent
Methanol	1.89±0.49
Ethanol	2.03±0.09
Chloroform	1.77±0.26
Petroleum ether	1.41±0.27
n- Hexane	1.55±0.25

*Values are the mean of duplicate experiments and represented as mean±SD*

## 4. CONCLUSION

After the screening of the phytoconstituents showed the presence of glycosides, carbohydrates, alkaloids, saponins, flavonoids and steroids. The n-hexane extract of the plant was found to possess the maximum (6.65 mg/gm) antioxidant capacity. The extracts revealed some total antioxidant capacity. On the other hand the methanol extract was found to contain highest (11.21 mg/gm) amount of flavonoids. From the results of the present study, it can be concluded that the plant extracts of *E. variegata* possess some antioxidant activities and this finding justifies its use as folk medicine.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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