Bangladesh J. Bot. **53**(3): 439-445, 2024 (September) DOI: https://doi.org/10.3329/bjb.v53i3.76217

# ANTIOXIDANT, MEMBRANE STABILIZING AND THROMBOLYTIC POTENTIALS OF THE LEAVES OF *ERYTHRINA FUSCA* LOUR.

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*Keywords: Erythrina fusca*, Antioxidant, Membrane stabilizing, Thrombolytic

## Abstract

*Erythrina fusca* Lour. (Family: Fabaceae) is famous for many traditional healing. In the present study, the leaves of *E. fusca* were collected, dried, powdered and then extracted with methanol. It was further partitioned into n-hexane, dichloromethane and aqueous soluble fractions. All the extracts were subjected to antioxidant, membrane stabilizing and thrombolytic assays. In case of free radical scavenging assay, the aqueous soluble fraction showed highest scavenging activity. In ferric reducing anti-oxidant power assay, the dicholoromethane extract showed highest reduction of ferric ion in a dose dependent manner. In total antioxidant assay, the methanol extract and dichloromethane extract showed stronger capacity as antioxidant. During the determination of total phenolic and flavonoid contents, the methanol extract and dichloromethane soluble fraction showed higher contents of phenolics and flavonoids, respectively. The erythrocyte membrane stabilizing assay revealed the capacities of methanol extract and its aqueous fraction to stabilize the RBC membrane in both hypotonic solution- and heat- induced hemolytic conditions. However, the clot lysis ability of the *E. fusca* leaf extracts was not so prominent.

## Introduction

South and Southeast Asia are blessed lands for nourishing varieties of medicinal plants due to their location around tropical areas. Many of the plants are known to be beneficial, but due to a want of proper systemic evaluation, their effects are not evident. In this circumstance, it is highly important to explore the medicinal qualities of these valuable plants. In human body, free radical can damage biochemical substances and produces oxidative stress. Vascular occlusion brought on by thrombus development in the circulatory system results in mortality. In the same time, inflammation is an issue for many decades as a notorious state. Many drugs are available for treating all these cases but not without side-effects. Bioactive molecules from plants might help to counter these issues (Akter *et al.* 2022).

*Erythrina* belongs to the family Fabaceae. Multiple alkaloids, triterpenes, sterols, stilbenes, phenols, coumarins, etc. were isolated earlier from *Erythrina* species (Rahman *et al.* 2007, Rahman *et al.* 2010). *Erythrina fusca* Lour. is a common tree in South and Southeast Asia. It is a deciduous tree having the height of 10 - 15 meters. Local inhabitants utilize this plant as antipyretic, anti-inflammatory, uterine stimulant, diurectics, wound healing agent, antifungal, antidote of snakebite, etc. (Abrescia and Golino 2005, Félix-Silva *et al.* 2017, Khan 2019, Sapura *et al.* 2020, Anjum *et al.* 2022). In the present study, *E. fusca* was selected for biological studies and subjected to antioxidant, membrane stabilizing (anti-inflammatory) and thrombolytic assays primarily.

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## Materials and Methods

The leaves of *Erythrina fusca* were collected from Fulbaria, Mymensingh, Bangladesh and a voucher specimen (DACB accession no. 45025) has also been deposited at Bangladesh National Herbarium, Mirpur, Dhaka, for future reference. The leaves were dried and then powdered. At room temperature for seven days, about 500 g of the powdered components were submerged in a volume of methanol measuring 1.5 liter. The methanol extract (EFLM) was concentrated and partitioned into n-hexane (EFLH), dichloromethane (EFLD) and aqueous (EFLA) soluble fractions (VanWagenen *et al.* 1993).

The free radical scavenging activity of the extract was assessed by using the stable free radical of 1,1 diphenyl-2-picrylhydrazyl (DPPH) (Kabir *et al.* 2010).

Different extracts of *E. fusca* leaf were treated with phosphate buffer, potassium ferricyanide and trichloroacetic acid to assess their ferric ion reducing potential (Afshari and Sayyed-Alangi 2017).

In case of total antioxidant capacity assay (Prieto *et al.* 1999), 1 ml of reagent (0.6 mol/l sulfuric acid, 28 mol/l sodium phosphate and 4 mol/l ammonium molybdate) were mixed with 0.1 ml of sample (200-25 µg/ml) in a tube to conduct the experiment. The total phenolic content of the extracts was determined by conducting an oxidation reaction using Folin-Ciocalteu reagent. In addition, the total flavonoid content was measured by using a colorimetric test with aluminium chloride (Ahmed and Rahman 2016).

Hypotonic solution- and heat- induced hemolysis of human erythrocytes models were used for membrane stabilization assay. In case of *in vitro* thrombolytic assay, venous blood was extracted from healthy participants, clot was made and treated with extracts (Akter *et al.* 2022). All of the extracts were put through a battery of phytochemical tests, i.e. alkaloids, triterpenoids, steroids and quinones (Gul *et al.* 2017).

Results were calculated as mean ± standard deviation (n=3) (Ahmed and Rahman 2016).

## Results and Discussion

Free radicals are responsible producing oxidative stress (Schetter *et al.* 2010). To counteract the problems, antioxidants are essential. Different extractives of the leaves of *E. fusca* were subjected to DPPH free radical scavenging, ferric reducing and total antioxidant assays.

In case of the DPPH free radical scavenging assay, the crude methanol extract and aqueous fraction of leaf displayed noticeable inhibition of free radicals (Fig. 1). The order of the reducing ability can be mentioned as: EFLA > EFLM > EFLD > EFLH. Reducing characteristics refer to ability of reductant to donate a hydrogen atom or an electron and therefore break a radical chain (Ramarathnam *et al.* 1995). Fig. 2A illustrates the $Fe^{+3}$ reduction power of various leaf extractives of *E. fusca*. According to the current findings, dichloromethane soluble fraction of methanol extract of leaf showed highest ferric reducing activity. The order of the activity can be mentioned as: EFLD > EFLM > EFLA > EFLH.

Phosphomolybdenum assay is very common to determine the total antioxidant capacity (Prieto *et al.* 1999). In the present study, the higher total antioxidant activity was demonstrated by both methanol extract and its dichloromethane fraction (Fig. 2B). The order of the activity can be mentioned as: EFLM, EFLD > EFLA > EFLH.

Phenolic compounds and flavonoids are excellent candidates as antioxidants. To know their level of existence, the total phenolic and flavonoid contents of the extractives were calculated (Fig. 3). Methanol extracts of leaf showed higher levels of phenolic contents (Fig. 3A). In case of

flavonoid contents, dichloromethane soluble fraction of methanol extract of leaf displayed noticeable levels of flavonoids (Fig. 3B).

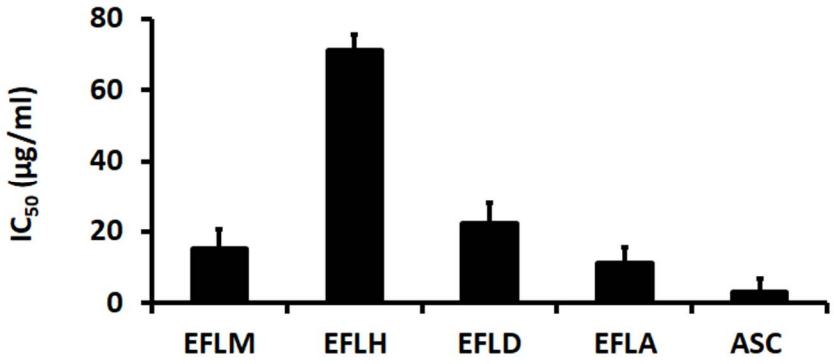


Fig. 1. DPPH free radical scavenging potentials of the leaf extracts of *Erythrina fusca*. EFLM: methanol extract of leaf, EFLH: n-hexane soluble fraction of methanol extract of leaf, EFLD: dichloromethane soluble fraction of methanol extract of leaf, EFLA: aqueous soluble fraction of methanol extract of leaf, ASC: ascorbic acid (standard drug), $IC_{50}$: Inhibitory concentration.

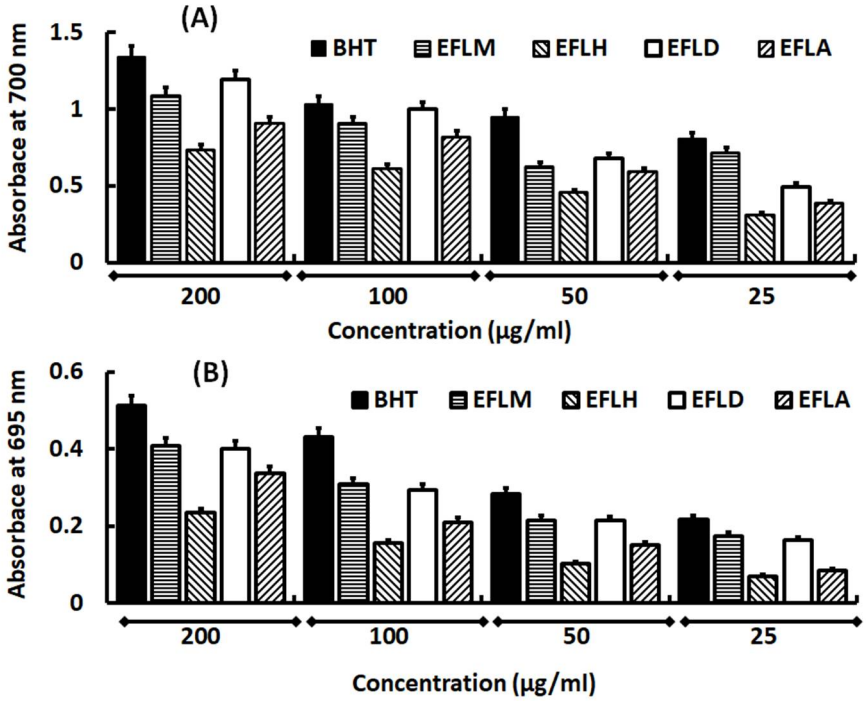


Fig. 2. Ferric reducing potential (A) and total antioxidant activity (B) of the leaf extracts of *Erythrina fusca*. BHT: butylated hydroxytoluene (standard). Other abbreviations are similar as in Fig. 1.

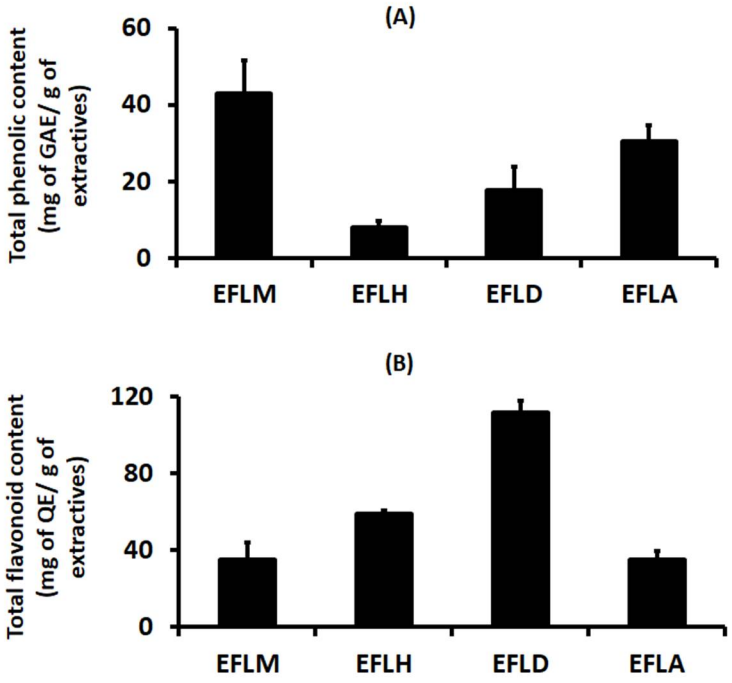


Fig. 3. Total phenolic contents (A) and total flavonoid contents (B) of the leaf extracts of *Erythrina fusca*. Abbreviations are similar as in Fig. 1.

Membrane stabilization assay can be used primarily as a tool to find out the anti-inflammatory materials. When erythrocytes are exposed to hypotonic medium, heat, methyl salicylate, phenylhydrazine etc., the lysis of the membranes occurs (Halliwell and Whiteman 2004) and free radical induced lipid peroxidation can aggravate the situation (Anosike *et al.* 2012). Membrane stabilization phenomena can prevent the inflammatory damages by preventing the leakage of serum protein and fluids into the tissues (Jean-Gilles *et al.* 2012). Methanol extract and aqueous fraction of leaf protected erythrocyte membrane from lysis strongly in hypotonic solution induced assay (Fig. 4A). In case of heat induced hemolysis, methanol extract showed highest level of inhibition of hemolysis (Fig. 4B).

The development of thrombus is associated with an increase in the number of vascular complications in human body (Hilleman and Campbell 2011). The leaf extracts were subjected to thrombolytic investigations but the extracts displayed weak thrombolysis compared to the standard (Fig. 5).

The result of the preliminary phytochemical screening of *E. fusca* plant is shown in Table 1. Steroids were present in n-hexane fraction to a large extent and moderately present in dichloromethane extract. However, triterpenoids were present in dichloromethane and aqueous fractions. Quinones were seen in dichloromethane fraction moderately. Numerous plant derived steorids and triterpenes were previously reported to contribute in anti-inflammatory activities (Ríos *et al.* 2000, Nunes *et al.* 2020). A lot of quinone derivatives were reported earlier to perform as antioxidant (Giner *et al.* 2022). Several alkaloids were seen previously to serve as anti-inflammatory and antioxidant agents (Souto *et al.* 2011, Sirin *et al.* 2023).

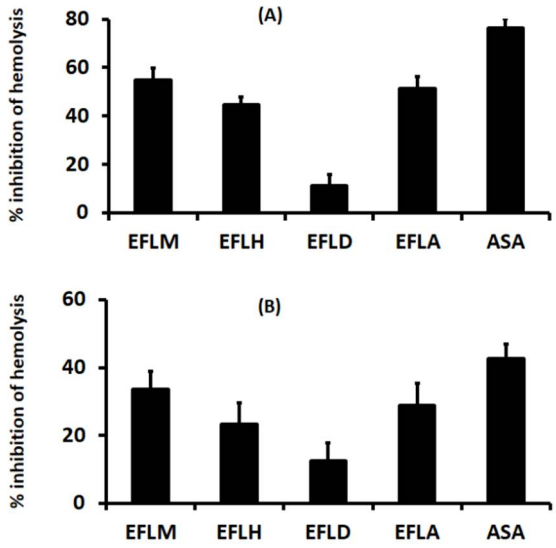


Fig. 4. Effect of different extracts of *Erythrina fusca* on hypotonic solution induced (A) and heat induced (B) hemolysis of erythrocyte membrane. ASA: acetylsalicylic acid (standard drug). Other abbreviations are similar as in Fig. 1.

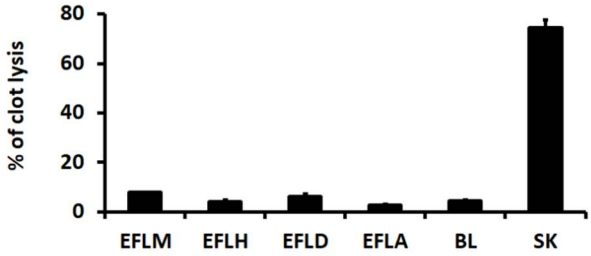


Fig. 5. Thrombolytic activity of the leaf extracts of *Erythrina fusca.* SK: streptokinase (standard drug), BL: blank. Other abbreviations are similar as in Fig. 1.

**Table 1. Preliminary phytochemical screening of *E. fusca.***

| Test | Crude methanol | n-hexane | Dichloromethane | Aqueous |
|---|---|---|---|---|
| Alkaloids | (++) | (-) | (++) | (+) |
| Triterpenes | (+) | (++) | ( ++) | (+ +) |
| Steroids | (++ ) | (+ + ) | (+ +) | (-) |
| Quinones | (+) | (-) | (+ +) | (+) |

+ indicates present at noticeable level, - indicates absent, ++ indicates higher level than +, +++ indicates higher level than ++.

In the present study, the leaves of *E. fusca* were extracted with methanol and partitioned into n-hexane, dichloromethane and water-soluble fractions. The extracts were subjected to antioxidant, membrane stabilizing and thrombolytic assays. Noticeable antioxidant and membrane stabilizing (anti-inflammatory) activities were seen in some extracts. However, thrombolytic activities were weak compared to the standard. Phytochemical profile indicated the presence triterpenes, alkaloids, quinones, phenolics and flavonoids.

**Acknowledgements**

This study was supported by the fund provided to MSR by the University Grants Commission of Bangladesh for conducting research in the University of Dhaka for the financial year 2021-2022 (Dhaka University /Reg/ admin-3/ 63258).

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*(Manuscript received on 10 January, 2024; revised on 31 July, 2024)*