

Assessment of Antioxidant, Antimicrobial, Cytotoxicity and Thrombolytic Potential of *Erythrina fusca* Lour Grown in Bangladesh

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Abstract

The current study was conducted to assess the antioxidant, antimicrobial, cytotoxicity and thrombolytic potential of the leaves and stem bark of *Erythrina fusca* Lour grown in Bangladesh. For the assessment of antioxidant properties, DPPH scavenging and phosphomolybdenum assays were used. In the DPPH free radical scavenging assay, the chloroform soluble fraction of a methanol extract of leaves of *E. fusca* exhibited the highest free radical scavenging activity with an IC₅₀ value of 5.5 ± 0.60 µg/mL, while the reference standard (ascorbic acid) showed an IC₅₀ value of 5.8 ± 0.18 µg/ml. All the test samples and the standard BHT demonstrated good linear relationship in the phosphomolybdenum assay. It was observed that the test samples contained considerable number of bioactive compounds including total phenolics in the aqueous soluble fraction of the leaves giving the highest 94.02 ± 1.004 GAE/100 g of dried sample. All test samples exhibited moderate antimicrobial activity against 13 Gram-positive and Gram-negative bacterial strains and 3 fungi, where the chloroform soluble fraction of the stem bark demonstrated the highest inhibition of growth with zone of inhibition of 19.3 ± 0.21 mm against *Bacillus cereus*. All the test samples displayed cytotoxic activity against brine shrimp nauplii having significant LC₅₀ and LC₉₀ values. The chloroform soluble materials demonstrated highest lethality with LC₅₀ value of 1.55 ± 0.30 µg/mL and LC₉₀ 65.68 ± 0.23 µg/ml. On the other hand, the carbon tetrachloride soluble fraction of the stem bark showed the highest clot lysis (22.75 ± 0.59%) as compared to that (66.77 ± 0.61%) revealed by reference standard, streptokinase. The findings of this study revealed that the leaves and stem bark of *E. fusca* growing in Bangladesh possesses bioactive compounds but further studies are required to purify and identify them.

Key words: *Erythrina fusca*, antioxidant, free radical scavenging, antimicrobial, cytotoxicity, thrombolytic.

Introduction

Erythrina is a genus of flowering plants in the pea family, Fabaceae. The generic name is derived from the Greek word (*erythros*), meaning "red," referring to the flower color of certain species (Gledhill, 2008).

Although some species show pink, purple, orange, white, green, yellow and even coral colors (Stein, 2009; Salma *et al.*, 2017). The genus has 290 species of which *Erythrina fusca* Lour is known as purple coral tree (Synonym: *E. atrosanguinea* Ridl., *E.*

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glauca Willd., *E. ovalifolia* (Roxb.). The tree is known as 'Manyā mandar, Kanta mandar or Harikakra'. It is a perennial, medium to large spreading tree 10-15 m tall with spines (1-2 cm) (Orwa et al., 2009). *E. fusca* is the most widespread species in the genus *Erythrina* that grows in both the Old and New World tropics (Ricardo et al., 1997). In Bangladesh, this plant is distributed in Chittagong and Cox's Bazar and mainly found along the swamps in main land (Flora of Bangladesh).

In various countries different parts of the plant *E. fusca* Lour have been used as traditional medicines. In Thailand, its root, bark and leaves are used as an antipyretic (Dasuki 2001), while the bark and leaves are utilized to relieve inflammation (Widianto 1980). The bark of this plant is used to relieve migraine in Peru (Valkenburg et al., 2001). The seeds are used to cure skin diseases while the inner bark is scraped for dressing fresh wounds in Indonesia (Singapore Botanic Garden, 2013).

A complete literature survey uncovered that most of the parts of the plant have been subjected assays for biological activities like anti-viral, anti-inflammatory, anti-tussive, rheumatism, hematuria, central nervous system depressant, hypotensive and as uterine stimulant (Widianto 1980; Singapore Botanic Garden, 2013; Wasuwat 1967; Russo et al., 1997; Mc Kee et al., 1997; Perry 1980; Ross et al., 1980; Ratnasooriya et al., 1999). Interestingly, the leaves and stem bark have not been subjected to assessment of antioxidant, antimicrobial, cytotoxicity and thrombolytic activities. In continuation of our phytochemical research the crude methanolic extract along with its *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions of the leaves and stem bark of *E. fusca* were assessed for its antioxidant, antimicrobial, cytotoxicity and thrombolytic activities in the present study.

Materials and Methods

Collection of plant materials and extraction: Fresh leaves and stem bark of *E. fusca* were collected from Nandail, Mymensingh District, Bangladesh in

September, 2011 and were authenticated in Bangladesh National Herbarium, where a voucher specimen has been preserved representing this collection (Accession No. DACB-35902). After adequate cleansing, the leaves and stem barks were cut into small pieces, at first air dried, then oven dried at relatively low temperature (30 °C) for 24 hours and finally pulverized into a coarse powder. The powdered leaves and stem bark (~800 g) was soaked separately in 2.25 L methanol for seven days, filtered through fresh cotton bed and finally with Whatman No. 1 filter paper, and concentrated by using a rotary evaporator at low temperature (36-40 °C) and reduced pressure. The concentrated crude methanol extracts (CME) of the stem bark and leaves were obtained as 30 gm and 15 gm, respectively.

The modified Kupchan partitioning protocol by VanWagenen et al. (1993) was used for the partitioning of an aliquot of the concentrated crude methanol extract (CME) of the stem bark and leaves of *E. fusca*. The consequent partitionates were dried off to yield *n*-hexane (HSF), carbon tetrachloride (CSF), chloroform (CHSF) and aqueous (AQSF) soluble fractions. The residues were then kept in a cool dry place until further use.

Determination of antioxidant activity: For the determination of antioxidant potential of the crude methanolic extract and its various soluble fractions 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay as developed by Brand-Williams et al. (1995) was followed.

Total antioxidant capacity: The total antioxidant capacity of the plant extracts was evaluated by the phosphomolybdenum method (Prieto et al., 1999) based on the reduction of Mo (VI) to Mo (V) by the extracts and subsequent formation of a green phosphate-Mo (V) complex in acidic condition.

Total phenolic content: The total phenolic content in the plant samples were determined spectrophotometrically by employing Folin-Ciocalteu reagent as oxidizing agent and gallic acid as the standard for equivalence (Agbor et al., 2014) as developed and described by Skerget et al. (2005).

Antimicrobial activity: The antimicrobial activity was performed using the agar disc diffusion method (Bauer *et al.*, 1966). Kanamycin (30 µg/disc) and griseofulvin (20 µg/disc) were used as reference standard against antibacterial and antifungal tests, respectively.

Cytotoxicity study: To ascertain the general toxic properties of the plant extractives the method established by Meyer *et al.*, 1982 and Rahman *et al.*, 2008a was employed. This method known as the brine shrimp lethality bioassay, is a useful method which indicates presence of bioactive compounds using dimethyl sulfoxide (DMSO) solutions of plant extractives against *Artemia salina* in a 1-day *in vivo* assay. As positive control vincristine sulfate was used. The logarithm of the sample concentration was used to calculate the median lethal concentration (LC₅₀).

Thrombolytic activity: The method developed by Prasad *et al.* (2006) and slightly modified by Kawsar *et al.* (2011) was used to evaluate the thrombolytic activity of all the test samples. Streptokinase was used as standard.

Statistical analysis: For statistical analysis of all the assays, three replicates of each sample were used and the values are reported as mean ± SD.

Results and Discussion

The main objective of our current study was to determine the antioxidant activity and total antioxidant capacity, total phenolic content, assessment the antimicrobial, brine shrimp lethality and thrombolytic activities of the crude methanolic extract of *E. fusca* as well as its soluble fractions. All the results have been illustrated in Tables 1-5.

The parameter generally used for the measurement of antioxidant activity was determined by the required concentration of the antioxidant to reduce the initial DPPH concentration by 50% (IC₅₀) (Sanchez-Moreno *et al.*, 1998). The IC₅₀ value is inversely proportional to the antioxidant power (Sethupandian *et al.*, 2017). The crude methanolic plant extract along with its modified Kupchan fractions of both the stem bark (IC₅₀ ≤ 12.1 µg/mL) and leaves (IC₅₀ ≤ 19.9 µg/mL) displayed significant

DPPH scavenging activity compared to the values obtained for ascorbic acid (IC₅₀ = 5.8 µg/mL) and tert-butyl-1-hydroxytoluene (BHT) (IC₅₀ = 24.35 µg/mL) as standards. All results are shown in Table 2.

Table 1. Modified Kupchan partitionates of the crude methanolic extract of *E. fusca* leaves and stem barks.

Crude extract/ soluble fractions	Leaves (g)	Stem bark (g)
CME	15	30
HSF	4.9	8.25
CSF	1.23	2.50
CHSF	0.95	2.00
AQSF	6.7	13.1

Phosphomolybdenum assay was used to determine the total antioxidant capacity of all the extractives expressing as the mg of ascorbic acid per gram of plant extract. The lowest total antioxidant capacity was shown by the chloroform soluble fraction of the leaves (106.10 ± 0.89 mg of ascorbic acid/g of dried extract) of *E. fusca* and highest was given by crude methanolic extract of the leaves (587.66 ± 0.85 mg of ascorbic acid/g of dried extract) as shown in Table 2. Plant polyphenols are effective as singlet oxygen scavengers, reducing agents and hydrogen atom donors (Karaman *et al.*, 2010; Rice-Evans *et al.*, 1996). That is why, it was logical to determine their total amount in the prepared extracts and fractions of *E. fusca*. Possible participation from other readily oxidized compounds in the plant materials and heterogeneousness of natural phenolics has led to introduction of several methods for determination of total phenolics. In most cases, Folin-Ciocalteu method has been found preferable as compared to the others (Singleton *et al.*, 1999). The amount of total phenolic content varied for the mother extract along with its different soluble fractions of the stem bark and leaves (Table 2) ranging from 8.82 ± 0.351 mg to 70.17 mg and 11.47 mg to 94.02 mg of Gallic Acid Equivalence (GAE)/100 g of dried extract, respectively. The highest total phenolic content was found from aqueous soluble fraction of

the leaves (94.02 ± 1.004 mg of GAE/100 g of dried extract) and the lowest in the carbon tetra chloride soluble fraction of the stem bark (8.82 ± 0.351 mg of GAE/100 g of dried extract). The crude methanolic extract along with its four soluble fractions were

screened for antimicrobial activity against five Gram-positive and eight Gram-negative bacteria and three fungi, and the findings are summarized in Table 3. For comparison purposes standard antibiotic discs of kanamycin and griseofulvin were used.

Table 2. Free radical scavenging, ferric reducing capacity, hydroxyl radical scavenging and total antioxidant activities of *E. fusca*.

Plant	DPPH free radical scavenging activity (IC_{50} μ g/ml)	Total antioxidant capacity (mg of ascorbic acid/gm of dry extract)	Total phenolic content (GAE/100 g of dry sample)
Stem bark			
CME	11.8 ± 0.35	472.21 ± 2.78	10.22 ± 0.505
HSF	7.7 ± 0.95	175.66 ± 0.63	46.11 ± 0.463
CSF	13.1 ± 0.75	125.99 ± 0.29	8.82 ± 0.351
CHSF	8.8 ± 0.95	216.54 ± 0.47	26.92 ± 0.829
AQSF	12.1 ± 0.45	415.10 ± 2.12	70.17 ± 0.463
Leaves			
CME	9.9 ± 0.95	587.66 ± 0.85	22.13 ± 0.617
HSF	7.9 ± 0.70	142.21 ± 0.52	11.47 ± 0.600
CSF	19.9 ± 0.95	106.10 ± 0.89	12.97 ± 0.189
CHSF	5.5 ± 0.60	260.77 ± 0.19	30.90 ± 0.223
AQSF	12.6 ± 0.20	153.77 ± 0.63	94.02 ± 1.004
Standard			
AA	5.8 ± 0.18	---	---
BHT	24.35 ± 0.02	---	---

During antimicrobial screening by the disc diffusion assay, all the plant samples of *E. fusca* at concentration of 400 μ g/mL exhibited varying degrees of antimicrobial activity (zone of inhibition = 12.1-19.3 mm) against all the test organisms (Table 3). The highest zone of inhibition was exhibited by the chloroform soluble fraction of the stem bark against *B. cereus* (19.3 mm). The manifestation of antibacterial activity of the crude methanolic extract along with its *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fraction against both Gram-positive and Gram-negative bacteria, and fungi may be indicative of the presence of broad-spectrum antimicrobial compounds. But more investigation is

required for proper utilization of this plant as a source of active antimicrobial principle(s).

In the brine shrimp lethality bioassay, cytotoxic compounds generally exhibit significant activity. Because of its simplicity and low cost, this assay can be recommended as a guide for the detection of antitumor and pesticide compounds (Mazid *et al.*, 2008). Also, this bioassay has a good correlation with the human solid tumor cell lines. As there is a correlation between cytotoxicity and activity against the brine shrimp nauplii using plant extracts thus the cytotoxic effect of the plant extracts can be selected for further cell line assay (Manilal *et al.*, 2009). In the case of brine shrimp lethality bioassay, a plot of percentage of the shrimps killed after 24 hours

against the logarithm of the sample concentration was used to calculate the median lethal concentration (LC₅₀) and 90% lethal concentration (LC₉₀). Regression analysis was used to obtain the best-fit line from the graph. The correlation (R²) between

concentration and mortality was 0.7997 to 0.9880. The chloroform soluble materials of the stem bark exhibited highest lethality with LC₅₀ value of 1.55 ± 0.30 µg/mL and LC₉₀ 65.68 ± 0.23 µg/ml (Table 4).

Table 3. Antimicrobial activity of stem bark and leaf extracts of *E. fusca* (400 µg/disc), kanamycin (30 µg/disc) and griseofulvin (20 µg/disc).

Test bacteria	Zone of inhibition (mm)										
	Stem bark					Leaf					PC
Gram-positive bacteria	CME	HSF	CSF	CHSF	AQSF	CME	HSF	CSF	CHSF	AQSF	Kan
<i>Bacillus cereus</i>	17.5± 0.40	16.9± 0.75	15.2± 0.55	19.3 ±0.21	14.2± 0.51	18.2± 0.83	17.9± 0.36	17.4± 0.91	15.8± 0.40	17.1± 0.72	35± 0.58
<i>B. megaterium</i>	18.1± 0.68	18.6± 0.70	14.9± 0.17	12.4 ±0.70	14.4± 0.74	17.1± 0.32	18.1± 0.72	15.7± 0.65	17.2± 0.72	15.4± 0.92	35± 0.58
<i>B. subtilis</i>	18.4± 0.75	18.3± 0.40	16.8± 0.36	17.6 ±0.42	14.8± 0.31	17.5± 0.53	16.9± 0.35	14.7± 0.35	16.1± 0.71	13.5± 0.32	36± 1.00
<i>Sarcina lutea</i>	18.3± 0.85	17.5± 0.32	16.6± 0.31	15.6 ±0.31	17.6± 0.31	18.4± 0.97	19.2± 0.15	16.2± 0.61	15.2± 0.51	15.6± 0.31	27± 0.58
<i>Staphylococcus aureus</i>	19.1± 0.74	18.1± 0.55	16.7± 0.45	17.4 ±0.42	15.8± 0.56	17.9± 0.44	17.6± 0.31	16.1± 0.55	15.1± 0.32	15.3± 0.55	32± 0.00
Gram-negative bacteria											
<i>Escherichia coli</i>	17.8± 0.78	16.9± 0.75	17.3± 0.60	17.7 ±0.66	18.3± 0.85	18.1± 0.72	17.8± 0.78	16.7± 0.40	15.1± 0.32	16.8± 0.64	25± 1.00
<i>Pseudomonas aeruginosa</i>	14.4± 0.35	13.4± 0.36	12.5± 0.69	12.1 ±0.87	12.7± 0.85	13.5± 0.25	12.1± 0.47	11.5± 0.15	12.8± 0.75	13.8± 0.25	20± 1.00
<i>Salmonella paratyphi</i>	19.2± 0.61	17.7± 0.56	17.6± 0.4	16.8 ±0.36	18.1± 0.9	17.9± 0.74	16.9± 0.7	14.7± 0.35	16.5± 0.35	17.3± 0.59	27± 0.58
<i>S. typhi</i>	16.2± 0.87	17.1± 0.21	16.8± 0.36	16.2± 0.81	15.4± 0.31	18.2± 0.42	17.4± 0.36	16.9± 0.55	16.8± 0.42	16.5± 0.35	22± 0.00
<i>Shigella boydii</i>	16.8± 0.36	17.8± 0.56	17.5± 0.31	16.5± 0.35	15.4± 0.31	17.3± 0.59	17.7± 0.56	15.9± 0.32	17.2± 0.47	16.9± 0.15	27± 0.58
<i>Sh. dysenteriae</i>	16.9± 0.45	18.1± 0.55	16.7± 0.35	15.8± 0.56	17.2± 0.31	16.5± 0.35	17.5± 0.29	16.1± 0.53	17.3± 0.59	13.8± 0.71	25± 0.58
<i>Vibrio mimiscus</i>	18.5± 0.53	17.6± 0.31	16.9± 0.15	15.8± 0.31	16.1± 0.53	17.8± 0.36	18.1± 0.55	16.2± 0.36	14.5± 0.40	13.8± 0.71	25± 0.58
<i>V. parahemolyticus</i>	15.8± 0.31	15.1± 0.35	15.5± 0.74	16.1± 0.85	15.6± 0.46	16.9± 0.15	17.9± 0.45	14.2± 0.15	15.1± 0.45	12.2± 0.70	20± 0.58
Fungus											
<i>Aspergillus niger</i>	14.4± 0.42	12.2± 0.87	13.7± 0.72	13.9± 0.57	13.5± 0.35	15.5± 0.51	14.5± 0.36	15.4± 0.31	12.3± 0.25	13.8± 0.71	20± 0.00
<i>Candida albicans</i>	15.1± 0.91	15.6± 0.31	16.1± 0.85	14.6± 0.64	12.5± 0.32	14.4± 0.35	13.8± 0.35	10.2± 0.46	12.2± 0.61	11.9± 0.61	18± 0.58
<i>Saccharomyces cerevisiae</i>	15.5± 0.38	12.5± 0.32	11.9± 0.72	13.2± 0.51	12.1± 0.74	14.1± 0.76	13.5± 0.15	13.5± 0.15	14.2± 0.30	12.8± 0.46	19± 0.58

CME=Methanol extract; HSF= n-hexane soluble fraction; CSF= Carbon tetrachloride soluble fraction; CHSF= Chloroform soluble fraction; AQSF= Aqueous soluble fraction; PC= Positive control, Kan = Kanamycin disc and Gri= Griseofulvin disc

Table 4. LC₅₀ and LC₉₀ values of the crude methanolic extract and its four soluble fractions in brine shrimp lethality bioassay.

		R ²	LC ₅₀ (µg/ml)	LC ₉₀ (µg/ml)
Standard				
Vincristine sulfate	30.799x + 60.645	0.9730	0.45 ± 0.11	10.0 ± 0.32
Samples				
Stem bark				
CME	y = 29.398x + 21.323	0.9280	9.45 ± 0.45	216.83 ± 0.43
HSF	y = 30.808x + 28.564	0.9573	4.96 ± 0.59	98.66 ± 0.30
CSF	y = 24.567x + 45.351	0.7997	1.55 ± 0.30	65.68 ± 0.23
CHSF	y = 30.005x + 29.566	0.8400	4.79 ± 0.65	103.31 ± 0.49
AQSF	y = 33.829x + 17.790	0.9719	8.95 ± 0.70	136.28 ± 0.47
Leaves				
CME	y = 31.413x + 22.810	0.9503	7.34 ± 0.40	137.70 ± 0.08
HSF	y = 34.636x + 18.788	0.9378	7.96 ± 0.19	113.77 ± 0.38
CSF	y = 28.996x + 25.824	0.9880	6.82 ± 0.67	163.41 ± 0.22
CHSF	y = 27.385x + 25.834	0.8243	7.63 ± 0.16	220.35 ± 0.33
AQSF	y = 34.232x + 19.292	0.9774	7.89 ± 0.52	116.29 ± 0.69

Table 5. Thrombolytic activity (% clot lysis) of the crude methanolic extract and its four Kupchan fractions.

	% Of clot lysis
Standard	
SK	66.77 ± 0.61
Blank	
Water	1.87 ± 0.38
Samples	
Stem bark	
CME	12.45 ± 1.31
HSF	14.27 ± 0.98
CSF	22.75 ± 0.59
CHSF	6.34 ± 0.47
AQSF	12.82 ± 0.35
Leaves	
CME	15.65 ± 0.94
HSF	15.33 ± 0.32
CSF	8.02 ± 1.78
CHSF	12.19 ± 1.07
AQSF	15.35 ± 0.35

In the circulatory system vascular blockage, sometimes leading to death is caused by thrombus formation. Thrombolytic agents that include tissue

plasminogen activator, urokinase, streptokinase etc. are currently used but these agents are still associated with risk of hemorrhage, anaphylactic reaction and also lack specificity. So, attempts are still ongoing around the world to develop improved thrombolytic agents (Wilson *et al.*, 2008; Rodriguez *et al.*, 2012; Sikder *et al.*, 2012). The crude methanolic extract and its *n*-hexane, carbon tetra chloride, chloroform and aqueous soluble fractions of both the stem bark and leaves of *E. fusca* revealed low to mild thrombolytic activity (Table 5). The carbon tetrachloride soluble fraction of the stem bark displayed the highest 22.75±0.59% clot lysis as compared to 66.77±0.61% clot lysis revealed by standard streptokinase. However, as negative control distilled water was used which gave a very minimal percentage of lysis of clot 1.87 ± 0.38%. The mean difference in clot lysis percentage between positive and negative control was found statistically very significant.

Conclusion

It is distinctly apparent from the above findings that the leaves and stem bark of *E. fusca* have significant free radical scavenging ability and

antioxidant properties. The plant has also exhibited mild to moderate antimicrobial potential, excellent cytotoxic and thrombolytic activities. Therefore, we can conclude that the plant *E. fusca* is a good candidate for further chemical investigation to isolate the bioactive constituents.

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