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### Article

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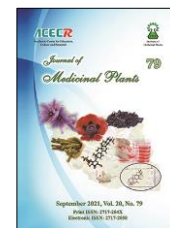
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### Research Article

## Flavonoid, pterocarpan and steroid from *Erythrina fusca* Lour. growing in Bangladesh: isolation, and antimicrobial and free-radical scavenging activity

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### ABSTRACT

**Background:** *Erythrina fusca* Lour. (fam. Fabaceae) is a flowering tree, found extensively in tropical and subtropical Asian countries, and is known for its use in traditional medicine for the treatment of various human ailments, for example, fever, liver complications, infections, and headaches. **Objective:** To carry out phytochemical study, and antimicrobial and free-radical scavenging activity evaluation of *E. fusca*. **Methods:** Ground stem bark of this plant was extracted by maceration with methanol, partitioned with various organic solvents, and compounds were isolated by chromatographic means. Structures of isolated compounds were confirmed by spectroscopic analyses. The antibacterial activity was assessed by the disc diffusion method, and the free-radical scavenging activity was determined by DPPH assay. **Results:** The carbon tetrachloride soluble fraction of the methanol extract of *E. fusca* afforded shinpterocarpin (1), lupinifolin (2), 3,9-dihydroxy-4-(3,3-dimethylallyl) [6aR,11aR]-pterocarpan (3) and  $\beta$ -sitosterol (4). Compounds 1-3 showed considerable antimicrobial activity against five Gram-positive and eight Gram-negative bacterial and three fungal strains tested in this study. Compound 1 exhibited the highest zone of inhibition of 19.4 mm against *Bacillus subtilis*. Additionally, compounds showed free-radical scavenging effects in DPPH assay with the IC<sub>50</sub> values of 8.8, 7.7 and 7.9  $\mu$ g/mL for compound 1, 2 and 3, respectively. However, they displayed some general toxicity in BSL assay. **Conclusion:** The isolation of bioactive compounds 1-3 supports some traditional medicinal uses of this plant. However, general toxicities found in the BSL assay might raise concerns regarding its safety, while offering a new avenue of future investigation on cytotoxicity of these compounds against human cancer cell lines.

**Abbreviations:** DPPH, 1,1-diphenyl-2-picrylhydrazyl; NMR, Nuclear Magnetic Resonance; BSL, Brine Shrimp Lethality; PTLC, Preparative Thin Layer Chromatography; CC, Column Chromatography

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## 1. Introduction

*Erythrina fusca* Lour (synonyms: *E. atrosanguinea* Ridl., *E. glauca* Willd., *E. ovalifolia* Roxb.; common name: Pannya mandar, Harikakra, Kanta madar; English: Coral bean and purple coral bean) (family: Fabaceae) is a perennial, and medium to large flowering tree with spreading spines (*i.e.*, 1-2 cm long, 10-15 m tall) [1-3]. This medicinal plant grows on the coasts and along rivers in tropical Asia (India, Sri Lanka, Myanmar, Indonesia), Australia, the Mascarene Islands, Madagascar, Africa and southern America. It also grows abundantly in Bangladesh.

In Thai traditional medicine, leaves, root and bark of *E. fusca* are used for its antipyretic potentials [3]. An infusion of the bark is used in the treatment of liver ailments and to induce sleep [4]. Decoctioned extract of the *E. fusca* bark is used in treating intermittent fevers like malaria [5]. The scraped inner bark is applied as a poultice on fresh wounds to prevent infections, and the stem and root bark mixture is used to heal violent and tenacious headaches. Bark and/or root decoctions are used in the treatment of beriberi [1-5]. The root is antirheumatic, sudorific and, in large doses, purgative. The bruised leaves are used for cleansing putrid ulcers, they are applied locally to treat the toothache, and the flowers have antitussive property. Decoctioned extract is used to soothe coughs.

Previous bioactivity studies on *E. fusca* revealed its antibacterial and antimalarial property [2], anti-estrogenic and estrogenic activity [1] and its potential in the treatment of ischemic-reperfusion injury [6]. Antiherpetic activity of the aerial parts of this plant was also reported [7].

A preliminary phytochemical screening of the aerial parts of *E. fusca* identified the presence of alkaloids, flavonoids, triterpenes, steroids, saponins, lactones, coumarins, reducing sugars, carotenoids, amines and cardiac glycosides [7]. Erythratidine was the first alkaloid (and first isolated secondary metabolite) isolated from *E. fusca* [8]. Later, several other compounds including erythrasinate [9] and other alkaloids [10], pterocarpan [11-13] and prenylated flavonoids [12, 14] were isolated from this species. The structure of the alkaloid epierythratidine was reassigned later, and further alkaloids were reported [15].

As a part of continuous explorations of bioactive phytoconstituents from the available medicinal plants of Bangladesh [16, 17], this study was designed to explore the phytochemical components from the stem bark of *E. fusca*, growing in Bangladesh, and evaluate the antimicrobial and free-radical scavenging properties as well as brine shrimp toxicity of the isolated compounds.

## 2. Materials and Methods

### 2.1. Plant materials

The stem bark of *E. fusca* was collected from Nandail, Mymensingh District, Bangladesh in September 2011 and identified by the Bangladesh National Herbarium, and a voucher specimen has been deposited there with the accession No. DACB-35902. The stem bark was sun-dried and cut into small pieces followed by pulverization.

### 2.2. Extraction, fractionation and isolation

The powdered material of *E. fusca* (800 g) was soaked in 2.25 L of methanol in a clean air-tight flat-bottom flask for five days at room

temperature with intermittent shaking. The methanolic extract was filtered initially through a fresh cotton-bed followed by Whatman No. 1 filter paper and subsequently concentrated by utilizing a rotary evaporator at reduced pressure and warm temperature (< 45 °C).

The crude methanolic extract (30 g) of *E. fusca* was subjected to fractionation through solvent-solvent partitioning process by using the modified Kupchan procedure [18] to obtain *n*-hexane (HxF, 8.25 g), carbon tetrachloride (CTF, 2.5 g), chloroform (CLF, 2 g) and aqueous (AqF, 13.1 g) soluble fractions. An aliquot of the CTF was subjected to further chromatographic separation with silica gel (70-230 mm, E-Merck, Germany)-packed column. The fraction was eluted initially with petroleum ether followed by petroleum ether/ethyl acetate and ethyl acetate/methanol gradients to obtain 250 sub-fractions (20 mL each). Afterwards, based on TLC profiles, the sub-fractions were bulked together to achieve concentrated products. Solvent evaporation from sub-fractions 90-97 afforded a yellowish mass, and further preparative TLC (mobile phase: toluene/ethyl acetate 9.7/0.3) yielded the compounds **1** (6.12 mg) and **2** (8.20 mg). The combined sub-fractions 117-127 were also purified through PTLC and gave compounds **3** (4.5 mg) and **4** (6.87 mg) using the mobile phases toluene/ethyl acetate 9/1 and 8.5 / 1.5, respectively. Both TLC and preparative TLC were run on pre-coated silica gel plates (60 mm, F<sub>254</sub> aluminum sheets, E-Merck, Germany). The plates were examined under the UV cabinet (at 365 and 254 nm) and vanillin/sulfuric acid reagent (1:100) was sprayed for proper visualization [19].

### 2.3. NMR analyses and characterization of compounds

<sup>1</sup>H-NMR spectra were recorded on a Bruker 500 MHz spectrometers, and the chemical shifts were reported with respect to the residual non-deuterated solvent signal. The structures of all compounds (**1-4**) were elucidated by the analysis of their <sup>1</sup>H NMR data and by direct comparison of these data with the respective published data.

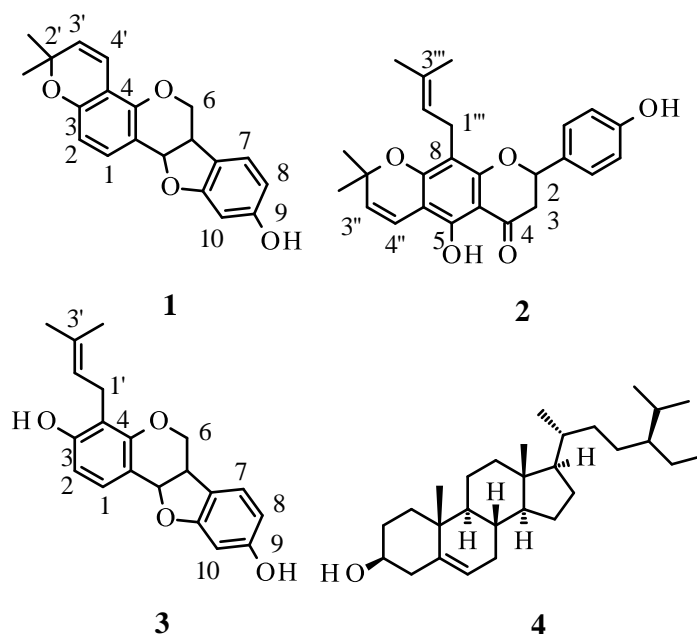
### 2.4. Bioassays

The brine shrimp lethality [20, 21], antimicrobial [22] and free-radical scavenging activities [23] of the isolated compounds were determined by the established methods. Griseofulvin and Kanamycin were used as the positive controls for antifungal and antibacterial assays, respectively. Ascorbic acid was used as the positive control for DPPH free-radical scavenging assay during the measurement of antioxidant potential of the reported isolated compounds. On the other hand, vincristine sulphate was used as a reference drug for brine shrimp lethality bioassay.

## 3. Results

### 3.1. Extraction, isolation and characterization

Four compounds including two pterocarpan shinpterocarpan (**1**) and 3,9-dihydroxy-4-(3,3-dimethylallyl) [6*aR*,11*aR*]-pterocarpan (**3**), a flavonoid lupinifolin (**2**) and a plant sterol  $\beta$ -sitosterol (**4**) (Fig. 1) were isolated from the CTF soluble fraction of the methanolic extract of the stem bark of *E. fusca* using the column chromatography and preparative thin layer chromatography (PTLC) techniques. The structures of the isolated compounds were elucidated by <sup>1</sup>H-NMR spectral data analysis (shown below) and comparison with published data.



**Fig. 1.** Structures of the isolated compounds, shinpterocarpin (**1**), lupinifolin (**2**), 3,9-dihydroxy-4-(3,3-dimethylallyl) [6aR,11aR]-pterocarpan (**3**) and  $\beta$ -sitosterol (**4**)

Shinpterocarpin (**1**): Oily transparent liquid;  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.43 (1H, d,  $J = 8.0$  Hz, H-1), 6.97 (1H, d,  $J = 8.0$  Hz, H-7), 6.57 (1H, dd,  $J = 8.0, 2.0$  Hz, H-8), 6.44 (1H, d,  $J = 2.0$  Hz, H-10), 6.52 (1H, d,  $J = 10.0$  Hz, H-4'), 6.36 (1H, d,  $J = 8.0$  Hz, H-2), 5.60 (1H, d,  $J = 10.0$  Hz, H-3'), 5.52 (1H, d,  $J = 11.0, 2.5$  Hz, H-11a), 4.81 (1H, br s, OH), 4.25 (1H, ddd,  $J = 11.0, 5.0, 2.5$  Hz, H-6 $\alpha$ ), 3.63 (1H, dt,  $J = 2.5, 11.0$  Hz, H-6 $\beta$ ), 3.50 (1H, m, H-6a), 1.45 (3H, s, 6'- $\text{CH}_3$ ) and 1.41 (3H, s, 5'- $\text{CH}_3$ ) [24].

Lupinifolin (**2**): Yellow needles;  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.25 (1H, s, OH-5), 7.33 (2H, d,  $J = 8.5$  Hz, H-2', H-6'), 6.87 (2H, d,  $J = 8.5$  Hz, H-3', H-5'), 6.63 (1H, d,  $J = 9.5$  Hz, H-4''), 5.50 (1H, d,  $J = 9.5$  Hz, H-3''), 5.34 (1H, dd,  $J = 12.5, 3.0$  Hz, H-2), 5.15 (1H, t,  $J = 7.0$  Hz, H-2'''), 3.22 (2H, d,  $J = 7.0$  Hz, H-1'''), 3.04 (1H, dd,  $J = 17.1, 12.5$  Hz, H-3 $\alpha$ ), 2.80 (1H, dd,  $J = 17.1, 3.0$  Hz, H-3 $\beta$ ), 1.66 (3H, s,  $\text{CH}_3$ -4'''), 1.57 (3H, s,  $\text{CH}_3$ -5'''), 1.46 (3H, s,  $\text{CH}_3$ -6''), 1.44 (3H, s,  $\text{CH}_3$ -5''), [12, 25, 26].

3,9-Dihydroxy-4-(3,3-dimethylallyl) [6aR, 11aR]-pterocarpan (**3**): Amorphous powder;  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.42 (1H, d,  $J = 8.5$  Hz, H-1), 6.97 (1H, d,  $J = 8.0$  Hz, H-7), 6.57 (1H, m,  $J = 8.0, 2.0$  Hz, H-8), 6.43 (1H, d,  $J = 2.0$  Hz, H-10), 6.39 (1H, d,  $J = 8.5$  Hz, H-2), 5.47 (1H, t like,  $J = 11.5$  Hz, H-11a), 5.29 (1H, s, OH-3/9), 5.27 (1H, d,  $J = 8.0$  Hz, H-2'), 4.80 (1H, s, OH-9/3), 4.25 (1H, dt,  $J = 5.0, 11.5$  Hz, H-6 $\alpha$ ), 3.65 (1H, dt,  $J = 5.0, 11.5$  Hz, H-6 $\beta$ ), 3.53 (1H, m, H-6a), 3.36 (2H, m, H-1'), 1.81 (3H, s,  $\text{CH}_3$ -4'), 1.75 (3H, s,  $\text{CH}_3$ -5') [27].

### 3.2. Brine Shrimp Lethality assay

In the Brine Shrimp Lethality assay, Compound **2** showed  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values of 3.17 and 62.95  $\mu\text{g/mL}$ , respectively. Compounds **1** and **3** exhibited  $\text{LC}_{50}$  values of 4.70 and 4.81  $\mu\text{g/mL}$ , respectively, and  $\text{LC}_{90}$  values of 129.12 and 86.90  $\mu\text{g/mL}$ , respectively. The positive control, vincristine sulphate, had the  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values of 0.45 and 10.00  $\mu\text{g/mL}$ , respectively.

### 3.3. Antimicrobial assay

Compounds **1-3**, at a concentration of 50 µg/disc, when subjected to antibacterial screening on traditional agar disc diffusion method, showed antimicrobial potentials with the zones of inhibition ranging from 14.4 to 19.4 mm against several Gram-positive and Gram-negative bacterial strains (Table 1). The largest zone of inhibition (19.4 mm) was observed for compound **1** against the Gram-positive bacterial strain of *Bacillus subtilis*. All tested compounds gave the zones of inhibition ranging from 11.9 to 15.1 mm in the case of antifungal test (Table 1), indicating comparable antifungal efficacy in

contrast to the standard antifungal drug, griseofulvin (18-20 mm).

### 3.4. DPPH assay

All three compounds (**1-3**) exhibited moderate to high free-radical scavenging activity in the DPPH assay [23]. The IC<sub>50</sub> (inhibitory concentration 50 %) values of compounds **1-3** were found as 8.8, 7.7 and 7.9 µg/mL, whereas the positive controls, *t*-butyl-1-hydroxytoluene and ascorbic acid, gave the IC<sub>50</sub> values of 24.35 and 5.80 µg/mL, respectively. The assay showed that compounds **1-3** could be similarly effective in scavenging free-radicals as the positive control ascorbic acid.

**Table 1.** Antimicrobial activity of compounds **1, 2** and **3** isolated from *E. fusca*.

| Tested bacteria               | Diameter of zone of inhibition (mm) |                            |                            |                              |
|-------------------------------|-------------------------------------|----------------------------|----------------------------|------------------------------|
|                               | Compound 1<br>(50 µg/disc)          | Compound 2<br>(50 µg/disc) | Compound 3 (50<br>µg/disc) | Kanamycin (30<br>µg/disc)    |
| <b>Gram-positive bacteria</b> |                                     |                            |                            |                              |
| <i>Bacillus cereus</i>        | 17.8 ± 0.66                         | 16.9 ± 1.10                | 19.0 ± 0.31                | 35                           |
| <i>Bacillus megaterium</i>    | 18.5 ± 0.87                         | 19.1 ± 0.60                | 15.6 ± 0.57                | 35                           |
| <i>Bacillus subtilis</i>      | 19.4 ± 0.31                         | 18.8 ± 0.35                | 14.9 ± 0.17                | 36                           |
| <i>Staphylococcus aureus</i>  | 17.6 ± 0.27                         | 18.1 ± 0.40                | 16.0 ± 0.42                | 32                           |
| <i>Sarcina lutea</i>          | 16.9 ± 0.68                         | 16.7 ± 0.60                | 17.6 ± 0.42                | 27                           |
| <b>Gram-negative bacteria</b> |                                     |                            |                            |                              |
| <i>Escherichia coli</i>       | 17.9 ± 0.21                         | 17.8 ± 0.27                | 17.4 ± 0.42                | 25                           |
| <i>Pseudomonas aeruginosa</i> | 14.8 ± 0.82                         | 14.8 ± 0.50                | 16.6 ± 0.21                | 20                           |
| <i>Salmonella typhi</i>       | 18.3 ± 0.85                         | 18.8 ± 0.42                | 17.3 ± 0.60                | 22                           |
| <i>Salmonella paratyphi</i>   | 17.5 ± 0.32                         | 17.8 ± 0.78                | 15.2 ± 0.51                | 27                           |
| <i>Shigella dysenteriae</i>   | 16.8 ± 0.36                         | 17.3 ± 0.59                | 15.9 ± 0.59                | 25                           |
| <i>Shigella boydii</i>        | 16.9 ± 0.55                         | 16.8 ± 0.42                | 17.7 ± 0.66                | 27                           |
| <i>Vibrio parahemolyticus</i> | 18.1 ± 0.55                         | 16.9 ± 0.15                | 17.9 ± 0.15                | 20                           |
| <i>Vibrio mimicus</i>         | 17.2 ± 0.31                         | 17.3 ± 0.40                | 16.5 ± 0.32                | 25                           |
| Tested fungi                  | Diameter of zone of inhibition (mm) |                            |                            |                              |
|                               | Compound 1<br>(50 µg/disc)          | Compound 2<br>(50 µg/disc) | Compound 3 (50<br>µg/disc) | Griseofulvin<br>(20 µg/disc) |
| <i>Aspergillus niger</i>      | 13.6 ± 0.31                         | 11.9 ± 0.32                | 13.2 ± 0.31                | 20                           |
| <i>Candida albicans</i>       | 14.2 ± 0.61                         | 13.6 ± 1.14                | 15.1 ± 0.35                | 18                           |
| <i>S. cerevisiae</i>          | 12.6 ± 1.10                         | 14.2 ± 0.60                | 14.2 ± 0.60                | 19                           |

#### 4. Discussion

CC and PTLC aided chromatographic separation of the CTF soluble fraction of the methanolic extract of the stem bark of *E. fusca* afforded the isolation of four compounds (**1-4**), and the structures of those compounds (except **4**) were deduced primarily based on <sup>1</sup>H NMR data analyses, and also comparison with respective published data. The identity of compound **4** was confirmed as b-sitosterol by co-TLC method. The <sup>1</sup>H-NMR spectrum of compound **1** showed four proton signals at 5.52 (1H, dd,  $J = 11.0, 2.5$  Hz) for H-11a, 4.25 (1H, ddd,  $J = 11.0, 5.0, 2.5$  Hz) for H-6 $\alpha$ ), 3.63 (1H, dt,  $J = 2.5, 11.0$  Hz) due to H-6 $\beta$  and 3.50 (1H, m) for H-6 which are indicative for a pterocarpan skeleton [24]. In the ring A, the presence of two doublets of aromatic protons at  $\delta$  7.43 (1H, d,  $J = 8.0$  Hz) for H-1 and  $\delta$  6.36 (1H, d,  $J = 8.0$  Hz) for H-2 indicated that C-3 and C-4 of the ring were substituted. In the D-ring, the doublets at  $\delta$  6.97 (1H, d,  $J = 8.0$  Hz) for H-7 and 6.44 (1H, d,  $J = 2.0$  Hz) for H-10 and a double doublet at 6.57 (1H, dd,  $J = 8.0, 2.0$  Hz) for H-8 demonstrated that the C-9 was substituted with a hydroxyl (-OH) group. The <sup>1</sup>H-NMR spectrum further showed two doublets ( $J = 10.0$  Hz) at  $\delta$  6.52 (1H) and 5.60 (1H) and two singlets of three proton intensity at  $\delta$  1.41 and 1.45 attributable, respectively, to the *cis*-double bond protons (H-4' and H-3') and *gem*-dimethyl groups at C-2' demonstrated the presence of a 2,2-dimethylchromene moiety. These <sup>1</sup>H-NMR data of compound **1** were found to be identical with the published values for shinpterocarpan [24]. Thus, compound **1** was identified as shinpterocarpan (**1**), which has never been reported from *E. fusca* before.

The <sup>1</sup>H-NMR spectrum of compound **2** revealed a sharp singlet at  $\delta$  12.25 (1H, s, OH-5) typical for the chelated hydroxyl group at C-5 in a flavonoid skeleton. The analytical features for a flavanone nucleus were evident from the proton signals at  $\delta$  3.04 (1H, dd,  $J = 17.1, 12.5$  Hz), 2.80 (1H, dd,  $J = 17.1, 3.0$  Hz) and 5.34 (1H, dd,  $J = 12.5, 3.0$  Hz), which could be assigned to H-3 $\alpha$ , H-3 $\beta$  and H-2, respectively of the flavanone nucleus for compound **2**. The doublet at  $\delta$  5.50 (1H, d,  $J = 9.5$  Hz, H-3'') and 6.63 (1H, d,  $J = 9.5$  Hz, H-4'') and two singlets at  $\delta$  1.44 (3H) and 1.46 (3H) were characteristic for the *cis*-double bond protons and *gem*-dimethyl groups of a 2,2-dimethyl-chromene moiety, respectively [27]. Two *ortho*-coupled doublets ( $J = 8.5$  Hz) centered at  $\delta$  7.33 (2H) and 6.87 (2H) were assigned to the protons at C-2' & C-6' and C-3' & C-5' of the *para*-disubstituted benzene ring (C ring). The singlets at  $\delta$  1.66 (3H, s, CH<sub>3</sub>-4''') and 1.57 (3H, s, CH<sub>3</sub>-5'''), a doublet at 3.22 (1H, d,  $J = 7.0$  Hz, H-1''') and a triplet at 5.15 (1H, t,  $J = 7.0$  Hz, H-2''') inferred the presence of an isoprenyl group. These <sup>1</sup>H spectral features of compound **2** were comparable with the NMR data of lupinifolin [25, 26] recorded in C<sub>6</sub>D<sub>6</sub>. Thus, compound **2** was identified lupinifolin (**2**), which has previously been isolated from the bark of *E. fusca* [12].

The <sup>1</sup>H-NMR spectrum of compound **3** displayed a set of proton signals at  $\delta$  3.65 (1H, dt,  $J = 5.0, 11.5$  Hz) and 4.25 (1H, dt,  $J = 5.0, 11.5$  Hz) assignable to H-6 $\beta$  and H-6 $\alpha$ , respectively another two proton resonances at  $\delta$  3.51 (m) due to H-6a and 5.47 (d,  $J = 6.5$  Hz) for H-11a. These spectral data suggested the existence of the -O-CH<sub>2</sub>-CH-CH-O- moiety connecting the rings B and C of the pterocarpan central skeleton.

Additionally, the  $^1\text{H-NMR}$  displayed a pair of *ortho*-coupled doublets ( $J = 8.5$  Hz) with one proton intensity at  $\delta$  7.42 for H-1 and 6.39 attributable to H-1 and H-2, respectively in ring-A, which demonstrated that C-3 and C-4 of the ring were substituted. The presence of three aromatic protons at  $\delta$  6.97 (1H, d,  $J = 8.0$  Hz, H-7), 6.57 (dd,  $J = 8.0, 2.0$  Hz, H-8) and 6.43 (1H, d,  $J = 2.0$  Hz, H-10) indicated an ABX spin system in ring D. Two broad signals were observed at  $\delta$  5.29 and 4.80, which could be assigned to the hydroxyl group protons. By comparing the  $^1\text{H-NMR}$  data of compound **3** with the published values [27], the structure of compound **3** was deduced as 3,9-dihydroxy-4-(3,3-dimethylallyl)[6a*R*,11a*R*]-pterocarpan. To the best of our knowledge, this pterocarpan has not previously isolated from *E. fusca*. It can be noted that this compound is biosynthetically related to compound **1**, as the isoprene unit on compound **3** is biosynthetically cyclized to form the pyran ring in compound **1**.

The presence of  $\beta$ -sitosterol (**4**), which is ubiquitously present in many plant species and one of the most common plant sterols, was established in *E. fusca* by running a co-TLC with the known-reference sample.

General toxicity of the compounds was determined by the brine shrimp lethality (BSL) assay [20, 21, 28] and the  $\text{LC}_{50}$  (lethal concentration 50 %) and  $\text{LC}_{90}$  (lethal concentration 90 %) values were acquired from the best-fit slope by plotting the concentration ( $\mu\text{g/mL}$ ) on x-axis and number of brine shrimp nauplii on y-axis for all tested compounds. Among the compounds, compound **2** was found to be the most toxic one ( $\text{LC}_{50}$  and  $\text{LC}_{90}$  values of 3.17 and 62.95  $\mu\text{g/mL}$ , respectively).

Compounds **1** and **3** were also toxic to brine shrimps to a lesser extent. However, none of the compounds were as toxic as the positive control, vincristine sulphate, which had the  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values of 0.45 and 10.00  $\mu\text{g/mL}$ , respectively. Vincristine is a well-known anticancer drug with established cytotoxicity and thus, its toxicity towards brine shrimps is expected to be high as well.

Although the preliminary antimicrobial activity of the crude extract of *E. fusca* was reported earlier [2], there is no previously published report on the isolation of antimicrobial compounds from this plant. Thus, the current finding, revealing considerable antimicrobial activity of compounds **1-3**, provides additional support and clarity to the previously published report, and establishes the compounds responsible, at least partially, for the antimicrobial property of the crude extract of *E. fusca*. It is noteworthy that all tested compounds exhibited significant antifungal property (the zones of inhibition ranging from 11.9 to 15.1 mm) which was comparable to antifungal efficacy in contrast to the standard antifungal drug, griseofulvin (18 - 20 mm).

The DPPH assay provides information on the ability of any test materials for scavenging free-radicals, and thus, reveals the potentials of those test materials as antioxidants. All three compounds (**1-3**) showed some degree of free-radical scavenging activity ( $\text{IC}_{50} = 8.8, 7.7$  and  $7.9$   $\mu\text{g/mL}$ , respectively) in the DPPH assay [23]. However, the  $\text{IC}_{50}$  value of the positive controls, *t*-butyl-1-hydroxytoluene and ascorbic acid, were 24.35 and 5.80  $\mu\text{g/mL}$ , respectively, which indicated that all three compounds were better than *t*-butyl-1-hydroxytoluene in terms of DPPH-scavenging ability, but were slightly less or similarly potent than ascorbic acid.



## 5. Conclusion

Among the isolated compounds, pterocarpan **1** and **3** have never been previously reported from *E. fusca*, or from the genus *Erythrina*. The isolation of bioactive compounds **1-3** potentially provides some scientific evidence in support of traditional medicinal uses of this plant. However, general toxicities found in the BSL assay might raise concerns regarding its safety, while offering a new avenue of future investigation on cytotoxicity of these compounds against human cancer cell lines.

## Author contributions

AA, MZS, SF and MKI generated data and compiled the first draft. MAR, LN and SDS provided the concept, prepared and edited the

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final manuscript, and are acting as corresponding authors.

## Conflict of interest

The authors declare that there is no conflict of interest.

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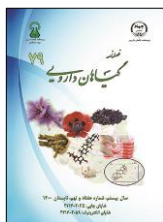
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## مقاله تحقیقاتی

ترکیبات فلاونوئیدی، پتروکارپانی و استروئیدی گونه‌ای گیاه فردوسی (*Erythrina fusca* Lour.) در حال رشد در بنگلادش: جداسازی و فعالیت ضد میکروبی و روبش رادیکال‌های آزاد  
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## چکیده

## اطلاعات مقاله

**مقدمه:** گیاه *Erythrina fusca* گونه‌ای فردوسی است که به‌طور گسترده در کشورهای گرمسیری و نیمه گرمسیری آسیا یافت می‌شود و به دلیل استفاده از آن در طب سنتی برای درمان بیماری‌های مختلف انسان، به عنوان مثال تب، اختلالات کبدی، عفونت‌ها و سردردها مشهور است. **هدف:** هدف از این تحقیق، بررسی فیتوشیمیایی و ارزیابی فعالیت ضد میکروبی و روبش رادیکال‌های آزاد گیاه *E. fusca* بود. **روش بررسی:** پوست ساقه آسیاب شده این گیاه با استفاده از متانول و روش خیساندن عصاره‌گیری شده و با حلال‌های مختلف آلی تقسیم بندی شده و ترکیبات به روش کروماتوگرافی جدا شدند. ساختار ترکیبات جدا شده با آنالیزهای طیف‌سنجی تأیید شد. فعالیت ضدباکتریایی با روش انتشار دیسک مورد بررسی قرار گرفت و فعالیت روبش رادیکال‌های آزاد با روش DPPH تعیین شد. **نتایج:** فراکسیون تتراکلرید کربنی عصاره متانولی گیاه دارای شینترپوکارپین (۱)، لویپنیفولین (۲)، ۹،۳-دی هیدروکسی-۴-(۳،۳-دی متیل آلایل) [Ra۱۱، Ra۶]- پتروکارپان (۳) و بتا- سیتوسترول (۴) بود. ترکیبات ۱ تا ۳ فعالیت ضد میکروبی قابل توجهی در برابر پنج باکتری گرم مثبت و هشت باکتری گرم منفی و سه سویه فارچی آزمایش شده در این مطالعه نشان دادند. ترکیب ۱ بالاترین هاله مهار رشد (۱۹/۴ میلی‌متر) را در برابر باسیلوس سوبتیلیس نشان داد. علاوه بر این، ترکیبات اثرات مهار رادیکال‌های آزاد را در روش DPPH با مقادیر IC<sub>50</sub> برابر با ۷/۷، ۸/۸ و ۷/۹ میکروگرم در میلی‌لیتر به ترتیب برای ترکیبات ۱، ۲ و ۳ نشان دادند. با این حال، آنها در تست سنجش عمومی سمیت BSL تا حدودی سمیت نشان دادند. **نتیجه‌گیری:** جداسازی ترکیبات فعال زیستی ۱ تا ۳ از برخی کاربردهای دارویی سنتی این گیاه پشتیبانی می‌کند. با این حال، سمیت عمومی یافت شده در روش BSL ممکن است نگرانی‌هایی را در مورد ایمنی آن ایجاد کند، در حالی که این تحقیق راه جدیدی از تحقیقات آینده در مورد سمیت سلولی این ترکیبات در برابر رده‌های سلولی سرطانی انسان ارائه می‌دهد.

کل واژگان:

گیاه فردوسی

فلاونوئیدها

پتروکارپان‌ها

استرول

فعالیت ضد میکروبی

سنجش DPPH

سنجش BSL

مخفف‌ها: DPPH، ۱، ۱- دی فنیل-۲- پیکریل هیدرازیل؛ NMR، رزونانس مغناطیسی هسته‌ای؛ BSL، کشندگی میگوی آب‌شور؛ PTLC، کروماتوگرافی لایه نازک جداکننده، CC، کروماتوگرافی ستونی

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