Phytochemical, biological and computational investigations of Erythrina fusca Lour. to assess antimalarial property against Plasmodium falciparum

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Abstract: For centuries medicinal plants have been traditionally used for prophylaxis and ailment of diseases. Nowadays it’s easy to isolate, purify, and characterize bioactive compounds with high efficacy. To investigate the medicinal especially antimalarial property of traditionally used plants, a number of Erythrina spp have been reviewed systematically where Erythrina fusca has been selected for further analysis. Phytochemical investigation included chromatographic separation and purification of compounds followed by characterization using NMR. In-vitro antimalarial drug sensitivity ELISA was carried out against chloroquine (CQ) sensitive 3D7 and resistant Dd2 strains. Additional biological tests such as central and peripheral analgesic, antioxidant, anti-diarrheal, hypoglycemic, thrombolytic, and membrane stabilization activities were also investigated. Molecular docking was performed using the isolated compounds against clinically important 14 Plasmodium falciparum proteins. For the first time, Phaseolin, Phytol, β-amyrin, Lupeol, and Stigmasterol are reported here and extracts showed significant antimalarial activity against 3D7 and Dd2 strains (IC50 4.94-22 µg/mL). Potent central analgesic, antioxidant and anti-diarrheal activities (p<0.05) and mild thrombolytic and membrane stabilization properties were also observed. Molecular docking of Phaseolin bolsters its potential as a new antimalarial drug candidate. This study projects significant medicinal values and necessitates further investigations to reveal its potential as a novel source of therapeutics.

Keywords: Antimalarial, Erythrina fusca, Phaseolin, Molecular docking, Phytochemical analysis.

1. Introduction

About 70% of the new drug molecules and their derivatives are of plant origin [1]. Chemical diversity of plants and their metabolites have contributed in the treatment of diseases like cancer, cardiovascular, liver, parasitic and viral infections, and even psychological disorders [2, 3]. Plants are rich in tannins, alkaloids, essential oils, glycosides, mucilage, and gums that play major vital roles in our physiological systems. Nevertheless, millions of plants are yet to be searched for their medicinal value. This study aims at phytochemical investigation of a plant through extraction,
isolation, characterization using chromatographic and spectroscopic techniques. The purpose includes biological investigations of the different fractions through bio-guided assays [4]. Depending on the characterization of the isolated compounds, in-silico ligand-protein interaction was also considered. On ethno-pharmacological point of view, Erythrina spp. a flowering plant in the family of Fabaceae, had been systemically reviewed. There are about 130 species in this family which are mainly distributed in tropical and subtropical regions all over the world. Erythrina fusca is one of the less investigated plants comparing other species of this genus and locally known as “Harikakra” in Bangladesh. E. fusca is a medium to large multi-branched, soft-wooded, spreading, deciduous tree usually ranging from 10-15m (max 30m). It is widely grown in tropical and Central America as a shade tree for cocoa and coffee as well as for ornamental purpose. Currently plants of this genus have been used for its antibacterial, anxiolytic, antinociceptive, antioxidant, antiplasmodial, etc. activities [5]. Therefore, it requires searching other potential properties as well as screening of medicinal values of unsearched species of this genus.

2. Results

2.1. NMR data:

**Phaseolin (1):** Orange crystals; 1H NMR (400 MHz, CDCl3): δ 1.45 (6H, s, H-7), 3.63 (1H, dd, J = 11.2 and 10.8 Hz, H-6a), 4.25 (1H, dd, J = 5.2 and 10.8 Hz, H-6b), 5.50 (1H, d, J=6.8Hz, H-18), 5.59 (1H, d, J=9.6Hz, H-14), 6.37 (1H, d, J=8.0Hz, H-10), 6.44 (1H, d, J=2.4Hz, H-4), 6.53 (1H, d, J=9.6Hz, H-15), 6.58 (1H, dd, J=8.4 and 2.4Hz, H-2), 6.44 (1H, d, J=2.4Hz, H-4), 6.98 (1H, d, J=8.0Hz, H-9), 7.44 (1H, d, J=8.4Hz, H-1).

**β-Amyrin (2):** Colourless mass; 1H NMR (400 MHz, CDCl3): δ 0.78 (3H, s, H3-24), 0.81 (3H, s, H3-28), 0.85 (3H, s, H3-29), 0.85 (3H, s, H3-30), 0.92 (3H, s, H3-32), 0.97 (3H, s, H3-26), 1.02 (3H, s, H3-27), 3.22 (1H, dd, J = 11.2, 4.8 Hz, H-3) and 5.21 (1H, t, J = 3.5 Hz, H-12).

**Lupeol (3):** Colourless mass; 1H NMR (400 MHz, CDCl3): δ 0.78 (3H, s, H3-24), 0.81 (3H, s, H3-28), 0.78 (3H, s, H3-24), 0.85 (3H, s, H3-25), 0.92 (3H, s, H3-27), 0.96 (3H, s, H3-23), 1.02 (3H, s, H3-26), 1.67 (3H, s, H3-30), 2.01 (1H, m, H-19), 3.22 (1H, dd, J = 11.2, 4.8 Hz, H-3), 4.58 (1H, br s, Ha-29), 4.71 (1H, br s, Hb-29).

**Stigmasterol (4):** Colourless crystals; 1H NMR (400 MHz, CDCl3): δ 0.69 (3H, s, H3-18), 0.79 (3H, d, J = 6.8 Hz, H3-26), 0.86 (3H, d, J = 6.8 Hz, H3-27), 0.91 (3H, d, J = 6.4Hz, H3-29), 0.99 (3H, s, H3-19), 1.01 (3H, d, J = 8 Hz, H3-21), 3.51 (1H, m, H-3), 5.01 (1H, dd, J = 15.2 and 8.4 Hz, H-23), 5.14 (1H, dd, J = 15.2 and 8.4 Hz, H-22), 5.34 (1H, d, J = 4.4 Hz, H-6).

**Phytol (5):** Colourless mass; 1H NMR (400 MHz, CDCl3): δ 0.89 (6H, d, J = 6.4 Hz, 7-CH3, 11-CH3), 1.08 (3H, d, J = 7.6Hz, 15-CH3), 1.59 (3H, s, 3-CH3), 1.08 (1H, m, H-7), 1.08 (1H, m, H-11), 1.59 (1H, m, H-15), 2.01 (1H, t, J = 6.8 Hz, H-4), 4.17 (1H, d, J = 6.8 Hz, H-1) and 5.43 (1H, t, J = 6.8 Hz, H-2).

2.2. Antimalarial activity:

The crude as well as the partitionates have high to moderate antimalarial activity against CQ-sensitive 3D7 and resistant Dd2 strains (IC$_{50}$ 4.94-22 µg/mL) as summarized in Table 1. The aqueous fractions exerted the most potent activity with IC$_{50}$ 4.94µg/mL against 3D7 strain. For the Dd2 strain, the n-hexane partitionates proved to be the most potent one with IC$_{50}$ 05µg/mL.
2.3 Antioxidant properties:

2.3.1 TPC test:
The total phenolic content of the partitionates were found between 73.87 to 164.16mg of gallic acid equivalent (GAE)/gm of extractives which is depicted in Table 2. The highest TPC was demonstrated by n-hexane partitionates with 164.84mg of GAE/gm of extractives.

2.3.2 DPPH Assay:
In the DPPH assay, the IC$_{50}$ values ranged from 14.02 to 57.25 (µg/ml) with the highest free radical scavenging activity found in the chloroform fraction (IC$_{50}$ 57.25 µg/ml) as shown in Table 2. For this assay, tert-butyl-1-hydroxytoluene (BHT) was used as standard with an IC$_{50}$ value of 50.13 µg/ml.

2.4 Thrombolytic and anti-inflammatory response:
Moderate thrombolytic activity was observed among the partitionates with chloroform fraction possessing the highest one (42.06 ± 0.33%). Streptokinase was used as standard with lysis of 69.99 ± 0.55% at 37°C and a minimal lysis was observed in the blank.

For anti-inflammatory response investigations, chloroform soluble fraction showed highest inhibition in hypotonic solution induced hemolysis with 86.19 ± 0.27% inhibition compared to 79.30 ± 0.93% inhibition in standard acetyl salicylic acid (ASA). In heat induced hemolysis method, aqueous fraction exerted 38.32 ± 0.92% inhibition compared to 43.70 ± 0.97% inhibition found in ASA. These results are found in Table 3.

2.5 CNS activity:
Statistical analysis indicated that both doses of crude extracts of E. fusca at 200mg and 400mg/kg body weight exerted extremely central analgesic effect after 30mins, 60mins and 90mins of administration as shown in Table 4.

2.6 PNS Activity:
The crude extract of E. fusca showed significant peripheral analgesic activity with high percentage inhibition of writhing both at 200mg/kg and 400mg/kg body weight. Thus it exposes significant peripheral analgesic activity which is depicted in Table 5.

2.7 Antidiarrheal Activity:
The methanolic crude extract of bark of E. fusca at both doses of 200mg/kg and 400mg/kg body weight showed moderate activity at the first and second hour of administration of the test compound. However, the activity decreased significantly at the third and fourth hours as shown in Table 6.

2.8 In silico studies:
High binding affinity were observed between the proteins and the ligand. Among the 14 proteins, pdb id 5jaz (-9.3kcal/mole), 5k8s (-8.8 kcal/mole) and 6ee4 (-8.7 kcal/mole) expressed excellent binding affinities which are described in Table 7 and Fig 1.
2.9. Figure and Tables

Figure 1. Ligand and clinically important protein high affinity interactions.

Table 1: Antimalarial activity of crude of *E. fusca* and its partitionates

| Fracns      | IC₅₀ values (µg/mL) | 3D7-102 Pf | Dd2-156 Pf |
|-------------|---------------------|------------|------------|
| Crude       | 13                  | 08         |
| n-Hexane    | 21                  | 05         |
| ChCl₃       | 22                  | 13         |
| Aqueous     | 5                   | 18         |
| Chloroquine | 27 nM               | 200 nM     |

Table 2: Antioxidant profiling of crude and different fractions

| Sample code | Total Phenolic Content (mg of GAE / gm of extractives) | DPPH Assay IC₅₀ (µg/ml) |
|-------------|--------------------------------------------------------|------------------------|
Table 3: Percent clot lysis, hypotonic solution and heat induced hemolysis by barks of *E. fusca*

| Sample code | Clot Lysis (%) | Hypotonic solution induced hemolysis | Heat induced hemolysis |
|-------------|----------------|--------------------------------------|------------------------|
| ME          | 23.18 ± 0.54   | 75.79 ± 0.33                        | 1.95 ± 0.65            |
| n-Hex       | 45.94 ± 0.73   | 57.30 ± 0.16                        | 31.88 ± 0.6            |
| ChCl₃       | 42.06 ± 0.33   | 86.19 ± 0.27                        | 27.42 ± 0.55           |
| AQ          | 32.21 ± 0.41   | 08.46 ± 0.25                        | 38.32 ± 0.92           |
| Blank       | 0.19 ± 0.13    | -                                    | -                      |
| STD         | 69.99 ± 0.55   | 79.30 ± 0.93                        | 43.70 ± 0.97           |

Table 4: Tail immersion effect of the crude extracts of bark of *E. fusca*

| Sample | After 30 min | After 60 min | After 90 min |
|--------|--------------|--------------|--------------|
|        | Average time of immersion (min) | Percent Elongation (%) | Average time of immersion (min) | Percent Elongation (%) | Average time of immersion (min) | Percent Elongation (%) |
| CTL    | 2.16 ± 0.19  | -            | 2.21 ± 0.42  | -            | 2.85 ± 0.37  | -            |
| STD    | 5.47 ± 0.16  | 153.20       | 9.50 ± 0.23  | 329.86       | 13.11 ± 0.48 | 360.00       |
| ME 200 | 3.87 ± 0.14  | 71.16        | 5.37 ± 0.69  | 142.99       | 6.62 ± 0.93  | 132.28       |
| ME 400 | 4.56 ± 0.27  | 111.1        | 6.45 ± 0.26  | 191.86       | 8.77 ± 0.39  | 207.72       |

Table 5: Percent inhibition of writhing in mice by crudes of *E. fusca*

| Sample code | Average writhing | Inhibition of writhing (%) |
|-------------|------------------|---------------------------|
| CTL         | 20.33 ± 1.53     | -                         |
| STD         | 03.33 ± 0.57     | 83.62                     |
| ME 200      | 11.33 ± 1.52     | 44.27                     |
| ME 400      | 8.00 ± 2.00      | 60.65                     |
Table 6: Antidiarrheal activity of crudes of bark of *E. fusca*

| Sample | 1st Hour | 2nd Hour | 3rd Hour | 4th Hour |
|--------|----------|----------|----------|----------|
|        | Average no. of faeces ± SD* | Inhibition (%) | Average no. of faeces ± SD | Inhibition (%) | Average no. of faeces ± SD | Inhibition (%) | Average no. of faeces ± SD | Inhibition (%) |
| CTL    | 2.67 ± 1.15 | - | 3.33 ± 1.14 | - | 1.33 ± 1.15 | - | 2.00 ± 1.00 | - |
| STD    | 0.00 ± 0.00 | 100.00 | 0.33 ± 0.57 | 90.09 | 1.33 ± 0.57 | 0.00 | 0.67 ± 0.57 | 66.50 |
| ME 200 | 1.33 ± 0.57 | 50.19 | 1.00 ± 0.57 | 69.97 | 0.33 ± 0.57 | 75.19 | 1.67 ± 0.57 | 16.50 |
| ME 400 | 0.33 ± 0.57 | 87.64 | 0.67 ± 0.57 | 79.88 | 1.00 ± 0.00 | 24.81 | 1.00 ± 1.00 | 50.00 |

*SD = standard deviation

Table 7: Binding affinity of ligand (Phaseolin) and 14 target proteins of *Plasmodium spp*:

| SL no | Protein-Ligand Complex | Binding Affinity | rmsd/ub |
|-------|------------------------|------------------|---------|
| 1     | 4plz_no_water_swiss_Conformer3D_CID_4063834 | -7.9 | 0 |
| 2     | 4qt2_no_water_swiss_Conformer3D_CID_4063834 | -7.8 | 0 |
| 3     | 4r1e__no_water_Conformer3D_CID_4063834 | -6.8 | 0 |
| 4     | 4r6w__no_water_swiss_Conformer3D_CID_4063834 | -8.5 | 0 |
| 5     | 4wi1__no_water_swiss_Conformer3D_CID_4063834 | -8.1 | 0 |
| 6     | 4zsg__no_water_swiss_Conformer3D_CID_4063834 | -8.3 | 0 |
| 7     | 5e16__no_water_swiss_Conformer3D_CID_4063834 | -8.2 | 0 |
| 8     | 5jaz__no_water_swiss_Conformer3D_CID_4063834 | -9.3 | 0 |
| 9     | 5k8s__no_water_swiss_Conformer3D_CID_4063834 | -8.8 | 0 |
| 10    | 5znv__no_water_swiss_Conformer3D_CID_4063834 | -7.9 | 0 |
| 11    | 6aqo__water_Conformer3D_CID_4063834 | -8 | 0 |
| 12    | 6ee4_no_water_swiss_Conformer3D_CID_4063834 | -8.7 | 0 |
| 13    | 6fbd__water_Conformer3D_CID_4063834 | -7.5 | 0 |
| 14    | 6i4b_no_water_swiss_Conformer3D_CID_4063834 | -7.5 | 0 |

3. Discussion

Eight compounds were isolated from *E. fusca*. The structures of the purified five compounds were determined through detailed analysis using nuclear magnetic resonance (NMR). Experiments consisted of proton (^1H-NMR), carbon (^13C NMR) as well as correlated spectroscopy (COSY), heteronuclear multiple bond coherence (HMBC), heteronuclear single quantum coherence (HSQC) NMR spectroscopic data and comparison with previously reported values. The remaining
Compounds (002, 9, 25, and 065) are being analyzed and their structures have yet to be confirmed. Among these, to the best of our knowledge, all compounds appear to be the first report from *E. fusca*.

**Phaseolin (1):** The $^1$H, $^{13}$C, HMBC, HSQC, COSY NMR spectrum (400 MHz, CDCl$_3$, Figure 1) of compound 017 displayed the presence of two methyl group from C-13 at $\delta$H 1.45 with $\delta$C 27.84 ppm with a HMBC correlation to $\delta$C 76.09 and 129.64 ppm. The $\delta$H 6.58 double doublet ($J = 8.4$ Hz & 2.4 Hz) was assigned to the H-2 with $\delta$C of 109.67 ppm. It has a HMBC correlation to $\delta$C 112.70 ppm which was assigned to C-19. $\delta$H 7.44 doublet ($J = 8.4$ Hz) was assigned to H-1 of the phenol ring in the structure with a HMBC correlation to $\delta$C 156.74 ppm. The other hydrogen H-4 with a coupling constant ($J = 2.4$ Hz) had been assigned with $\delta$H 6.45 ppm doublet. The H-6a and H-6b have shown double doublet in the spectrum due to two non-equivalent proton in the same and adjacent carbon molecule with $\delta$H 3.63 and 4.25 ppm. The higher ppm is due to methoxy group and the HSQC correlation confirmed the same carbon with a chemical shift $\delta$C 66.69 ppm. The multiplet of 3.52 ppm would be assigned to H-7 in the dihydropyran ring fused with a dihydrofuran ring structure. The H-9 and H-10 were confirmed with ortho-coupling constant ($J_{ ortho } = 8$ Hz) with a chemical shift of 6.98 doublet and 6.37 doublet respectively. Another ortho-coupling ($J = 9.6$Hz) of the pyran ring with chemical shift of 5.59 doublet and 6.53 doublet were confirmed to H-14 and H-15 position respectively with HSQC data correlating to $\delta$C 129.64 and 116.52 respectively. The HMBC correlation of H-14 and H-15 were 106.25 and 76.09 ppm that were assigned to C-16 and C-12 respectively. Finally, the H-18 of the fused dihydropyran and dihydrofuran ring was assigned a chemical shift of 5.5 doublet with $\delta$C of 78.7 ppm. All these sub structures impose a chemical structure that has a resemblance only with phaseolin.

**β-amyrin (2):** The $^1$H NMR spectrum (400 MHz, CDCl$_3$, Figure 2) of 001 (mixture) exhibited the presence of triterpenoid carbon skeleton, an oleanane-type, comprising of eight methyl singlets at $\delta$ 0.92, 0.78, 0.97, 0.97, 1.02, 0.81, 0.85 and 0.85 which had been assigned to Me-23, Me-24, Me-25, Me-26, Me-27, Me-28, Me-29 and Me-30. A double doublet of a single proton intensity at $\delta$ 3.22 ($J = 11.2$, 4.8 Hz) implied a pentacyclic triterpene oxymethine proton (H-3). Moreover, a triplet at $\delta$ 5.21 ($J = 3.6$ Hz) was assigned to the proton at C-12 suggesting a skeleton of the olean-12-ene-form. Hence, 001 was characterized as β-amyrin by comparing its spectral data to reported values in addition to co-TLC with genuine sample [6].

**Lupeol (3):** $^1$H NMR spectrum (400 MHz, CDCl$_3$, Figure 3) of 001 also displayed a double doublet ($J = 11.2$ Hz and 4.8 Hz) of a single proton intensity at $\delta$ 3.22, indicating a proton of oxymethine group (C-3) in a triterpene type carbon skeleton. β orientation of the C-3 oxygenated substituent can be confirmed by coupling constant as well as splitting pattern of this proton. Vinylic protons at C-29 could be attributed by two broad singlets at $\delta$ 4.71 and 4.58 (1H each). The 1H NMR spectrum showed a characteristic multiplet of a single proton peak at $\delta$ 2.01 attributable to the proton at C-19 and six singlets at $\delta$ 0.96, 0.78, 0.85, 1.02, 0.92 and 0.81 (3H each) ascribed to methyl group protons at C-4 (H$_3$-23, H$_3$-24), C-10 (H$_3$-25), C-8 (H$_3$-26), C-14 (H$_3$-27), and C-17 (H$_3$-28) respectively. The downfield methyl group resonance at $\delta$ 1.67 could be assigned to the vinylic methyl at C-20 (H$_3$-30). Thus, MNC-3 was characterized as lupeol. Further confirmation was performed by comparing its $^1$H NMR data to the published values as well as by co-TLC [7].
Stigmasterol (4): The 1H NMR spectrum (400 MHz, CDCl₃, Figure 4) of 31 exhibited signals indicative of a steroidal compound. A multiplet at δ 3.51 of one proton intensity implied a steroidal nucleus at H-3. A single proton doublet at δ 5.34 (J = 4.4 Hz) indicated the olefinic H-6 of the steroidal nucleus. Moreover, olefinic protons were displayed at δ 5.14 and 5.01 (H-22 and H-23). Two singlets at δ 0.69 and δ 0.99 (3H each) indicated two tertiary methyl groups at C-13 (H₃-18) and C-10 (H₃-19), respectively. Two methyl groups at C-25 showed two doublets (J = 6.8 Hz) at δ 0.79 (H₃-26) and 0.86 (H₃-27). Another doublet (J = 8.0 Hz) at δ 1.01 (H₃-21) could be ascribed to methyl group at C-20. Additionally, the doublet (J = 6.4 Hz) at δ 0.91 integrated for three proton indicated the primary methyl attached to C-28 (H₃-29). The above spectral features have resemblance for stigmasterol that was further confirmed by Co-TLC with original sample [8].

Phytol (5): The 1H NMR spectrum (400 MHz, CDCl₃, Figure 5) of 003 showed a doublet at δ 4.17 (J = 6.8 Hz) integrated for two protons could be assigned to H-1. At δ 2.01, a triplet of two proton intensity indicated the protons at H-4. The olefinic methine (=CH-) could be deduced by a single proton peak at δ 5.43 with a broad triplet. A multiplet at δ 1.07 could be ascribed to protons at H-7 and H-11. The proton attached to the terminal methyl at C-15 was deduced by a multiplet at δ 1.66. A doublet observed at δ 0.89 was assigned to the six methyl protons positioned at C-7 and C-11. Moreover, doublet at δ 1.08 (J = 7.6 Hz) was ascribed to H₃-16 and H₃-17 (C-15) for another six protons of methyl groups. These features were similar to those of phytol and therefore, 003 was characterized as a diterpene alcohol Phytol [9].

There are many species in the Erythrina genus that had been extensively studied for different biological activity and diversified medicinal values. The species investigated in this studies is also found throughout the world [10]. Bactericidal effects had been reported in the leaves aquadest extract of the E. fusca [11]. Other than antibacterial effect, it is also effective against malaria parasites [12]. Subal et. al experimented on the antiepileptic and antioxidant property of the hydro-alcoholic extract of bark of E. fusca [13, 14]. The antiepileptic effect noticed might be due to the enhancing of the gamma amino butyric acid (GABA) [13] and they also found an increase in the superoxide dismutase activity and reduced glutathione levels as well as reduced level of malondialdehyde (MDA) that may be due to its antioxidant property [14]. In our study, we performed DPPH assay and determined the total phenolic content and observed significant antioxidant properties of the crude and its partitionates of the bark of E. fusca. In a study conducted by Khaomek et al. found antimalarial property in the ethyl acetate extract of the stem bark and prenylated flavonoid isolated from the E. fusca against multi-drug resistant Pf strain K1 [12]. However, in that study the stem bark of E. fusca had not been used for medicinal resources and only three of the isolated compounds expressed activity at concentration <12.5µg/ml [12]. In comparison, we performed antimalarial assay of the crude extract of the bark of E. fusca and its different Kupchan partitionates against CQ sensitive 3D7 and resistant Dd2 strain and observed significant antimalarial effect. Here, IC₅₀ 4.94µg/mL and 05µg/ml were obtained in aqueous fractions and n-hexanes fractions against 3D7 and Dd2 strains respectively. Their antimalarial activity was categorized as: high (IC₅₀<5 µg/mL), promising (5< IC₅₀<15 µg/mL), moderate (15< IC₅₀<50 µg/mL) and inactive (IC₅₀>50 µg/mL) based on previous reports.[15-17]

Other than the aforementioned biological activities, significant central analgesic activity has been observed through high percentage of elongation time in mice (71.16-207.72%) after 30, 60 and
90 minutes. Significant inhibition of writhing (60.65%) was observed by crude extract at 400mg/kg body weight dose. The antidiarrheal activity tested in this study suggests moderate activity within the 1st and 2nd hour (79.88-87.64% at 400mg/kg dose) of administration of the test compound. Activity decreased significantly at the 3rd and 4th hours. This might be due to short duration of action or high clearance rate. The percent clot lysis and inhibition of hemolysis demonstrate moderate thrombolytic and anti-inflammatory property of the crude and its partitionates. Though eight compounds were isolated from *E. fusca*, none of them could be tested for any biological activities due to inadequacy of samples.

Virtual screening through docking via AutoDock Vina against shortlisted 14 antimalarial drug proteins sorted from 787 proteins with crystallographic structures *in silico* revealed excellent binding affinity of the isolated and 3D simulated compound Phaseolin. The 5jaz protein is associated with parasite growth, 5k8s is linked with parasite invasion in red blood cells (RBC), and 6ee4 plays major role in *P. falciparum* and *P. vivax* by hemoglobin digestion. There is an urgent clinical need for new inhibitors and molecular docking imposes significant potential of the compound Phaseolin as a future antimalarial drug candidate against these drug targets.

4. Materials and Methods

4.1 Chemical Investigation:

4.1.1 Plant Collection:

Bark was collected in August 2018 from Dhaka Botanical Garden. After collection, taxonomical identification was done by the Bangladesh National Herbarium. A sample specimen has been deposited with ID- DACB accession number 48256.

4.1.2 Preparation of Plant Extract and Fractions:

The bark was shade dried at rooftop for a week and ground to a coarse powder. Subsequently, 500gm dried coarse powder was soaked in 2L of methanol for 2 weeks with regular frequent stirring for the extraction of compound(s) through cold maceration. The filtrate was then collected through multiple filtrations with cotton plug, Whatman no. 1 filter paper as well as decantation and evaporated to dryness using rotary evaporator (*Büchi*® rotary evaporator Model R-200). The fractionation was done using slightly modified version of Kupchan method of solvent-solvent partitioning [18] and yielded three partitionates; n-hexane, chloroform, and left over mother methanolic aqueous fraction which were stored in 4°C in aliquots of different concentration range (10-100µg/mL) for further biological activity analysis.

4.1.3 Size Exclusion Chromatography:

Three gm of n-hexane and chloroform extracts were subjected to size exclusion chromatography using lipophilic Silica 60G and later 600mg of similar fractions were subjected to Sephadex (LH-20). Gradient solvent system of n-hexane, ethyl acetate, and methanol were used resulting in 48 different fractions. For the Sephadex column, the solvent system was n-hexane: chloroform: methanol (2:5:1) initially and then 10% methanol in chloroform with gradual increase by 10% up to 100% methanol. The preparative thin layer chromatography (PTLC) was performed on several fractions based on their TLC behavior. Eight of the purified compounds, four from n-hexane and four from chloroform partition, were spectroscopically analyzed.
4.2 Biological Activity:

4.2.1 Antimalarial Activity:

(i) Malaria culture maintenances:

In this study, both chloroquine(CQ) sensitive *Plasmodium falciparum* (*Pf*) strain 3D7 (MRA-102) and CQ-resistant Dd2 (MRA-156) strains were used from BEI-resources (MR4/ATCC® Manassas, VA, USA). The continuous *in-vitro* culture of asexual *Pf* blood stage was maintained as described by Trager and Jensen with slight modification [19].

(ii) Evaluation of *in-vitro* antimalarial activity:

Antimalarial assay was performed using HRPII based enzyme-linked immunosorbent assay (ELISA) method described by Noedl *et al.* and WWARN [20-22]. The data is analyzed using GraphPad Prism 8.2 (La Jolla, CA 92037 USA) to construct a non-linear regression graph fit with a sigmoidal dose-response curve and determine the inhibitory concentration (IC$_{50}$) value for individual samples.

4.2.2 Antioxidant activity:

(i) Total Phenolic Content (TPC) determination:

The Folin-Ciocalteu method is considered as one of the reference methods for determination of the total phenolic content (TPC). The phenolic content in the extracts were determined using method established by Harbertson and Spayd [23].

(ii) DPPH Test:

The antioxidant capacity of the plant extracts was assessed by the technique of Brand-Williams *et al.* using 1,1-diphenyl-2-picrylhydrazyl (DPPH) [24].

4.2.3 Anti-inflammatory response:

The efficacy of drugs in respect of stabilization of erythrocytes can be deduced by the lysosomal membrane stabilization method as the human red blood cell membranes have deep resemblance with the lysosomal membrane. Extractives were evaluated for capacity to inhibit both hypotonic solution and heat induced hemolysis [25].

4.2.4 Thrombolytic Activity:

Thrombolytic capacity was analyzed using method demonstrated by Prasad *et al.* [26].

4.2.5 Central Analgesic Activity:

Central nervous system activity was evaluated using immersion of tail method. For this purpose, a constant heat of 56°C is applied to the test animal i.e. the rat-tail. The heat acts as a pain stimulus and change in sensitivity occurs due to analgesic activity of the test compounds. The rat shows quick withdrawal of tail when the stimulus exceeds the threshold. The time elapsed from the immersion to the deflection was recorded for each group and their average calculated. Percentage of elongation of tail immersion time was calculated in respect to control [27].

4.2.6 Peripheral Analgesic Activity:

Acetic acid induced abdominal writhing method was used to evaluate the analgesic activity of the test extracts [28].
4.2.7 Anti-diarrheal Activity:
The method demonstrated by Shoba and Thomas was used for the assessment of the antidiarrheal activity of the test compound [29].

4.3 Molecular Docking Calculations:

**Conformer3D_CID_4063834 (Phaseolin)** was subjected to molecular docking study against various proteins of *Pf* (4plz, 4qtl2,4r1e, 4r6w, 4wi1, 4zxa, 5e16, 5jaz, 5k8s, 5znc, 6aqs, 6ee4, 6fba and 6i4b pdb id). AutoDock Vina software was employed for flexible docking and docking calculations [30]. Crystal structures were obtained from the Protein Data Bank (PDB) and prior to docking ligand, water molecule removal and necessary energy minimization was performed. The centre grid box was positioned to cover all the active sites in x, y and z directions and torsional rotation was allowed for optimal flexible docking (see Fig 1).

5. Conclusions
Repeated chromatographic separation and purification of the barks of *Erythrina fusca* Lour. (family: Fabaceae) afforded eight compounds; among which five were identified. The extractives of *E. fusca* revealed significant antimalarial, central analgesic, and antioxidant activities and moderate anti-diarrheal, thrombolytic and membrane stabilization properties. Moreover, molecular docking of the 3D simulated compound phaseolin *in silico* expressed excellent binding affinity towards clinical antimalarial drug target in virtual screening via AutoDock Vina that indicates its potential as future antimalarial drug. Therefore, the plant materials can be further investigated extensively to find out its unexplored efficacy that may be helpful in the discovery of a new lead compound.

**List of abbreviations**

CQ chloroquine

*Pf* *Plasmodium falciparum*

ELISA enzyme-linked immunosorbent assay

IC50 inhibitory concentration

TPC total phenolic content

DPPH 1,1-diphenyl-2-picrylhydrazl

PDB Protein Data Bank

GAE gallic acid equivalent

BHT tert-butyl-1-hydroxytoluene

ASA acetyl salicylic acid

1H-NMR proton nuclear magnetic resonance

13C NMR carbon nuclear magnetic resonance

COSY correlated spectroscopy

HMBC heteronuclear multiple bond coherence

HSQC heteronuclear single quantum coherence

GABA gamma amino butyric acid

MDA malondialdehyde

RBC red blood cells

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MSA conceived the concept and coordinated the study, supervised the study, and revised the manuscript. All authors read and approved the final manuscript.

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