



## Antibacterial Activity and Phytochemical Screening of *Erythrina fusca* Lour. Leaf Extract (Fabaceae)

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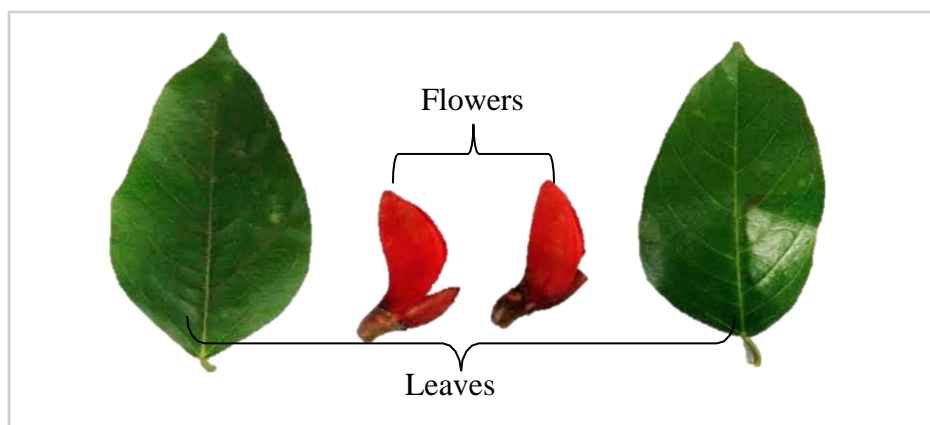
### ABSTRACT

*Erythrina fusca* Lour. (Family Fabaceae) is a flowering tree, locally known as ‘Chengkering’. The plant is traditionally used in the treatment of some symptoms related to bacterial infections such as skin itching and inflammations as well as on wounds. This work reports for the first time on the *in vitro* antibacterial activity of methanolic leaf extract of *E. fusca* against some common bacterial strains such as *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus aureus*. The *in vitro* antibacterial assay was carried out using agar well diffusion method with the extract concentration of 3 mg/well and vancomycin 30 µg/well as the positive control. Except for *K. pneumonia*, it was found that the extract exhibits antibacterial effects against all the tested bacterial strains with the zone of inhibition (ZOI) in a range of 10.5 – 14 mm. Based on the preliminary phytochemical screening using standard qualitative phytochemical tests, the leaf extract contains a significant number of alkaloids, flavonoids, terpenoids, and tannins, which might contribute to its antibacterial activity. Both antibacterial properties and the presence of various phytochemicals in the extract could support the traditional uses of *E. fusca* in treating symptoms related to bacterial infections. The findings would serve as a basis for further exploration of the antibacterial potential of the plant’s leaves.

**Keywords:** Medicinal plant, bacterial infections, antibacterial activity, phytochemical screening, alkaloids

## INTRODUCTION

Malaysia is one of the world's 12 megadiverse countries, with the greatest levels of endemism. Many trees flora is also an important source of medicinal natural products including the plants in genus *Erythrina* [1]. *Erythrina* belongs to the legume family (Fabaceae). The name "*Erythrina*" is originated from the Greek word "erythros" which means "red" referring to the red colour of the plant's flowers [2-4]. To date, around 290 species of *Erythrina* have been reported, where seven are available in Malaysia including *E. fusca*, which is locally known as "Chengkering" [5-8]. *E. fusca* can be described morphologically as a deciduous tree 10-15 m tall. The barks structure of this species has brownish-grey flaky, while its leaves are leaflets ovate to elliptical (2.5-20 cm x 1.5-15 cm), sub-coriaceous, rounded, or subacute at both ends, pale green above, glaucous, or greyish green beneath, glabrous to velvety hairy. The red flowers of this plant have campanulate calyx, about 1.5 m long [5]. Figure 1 below shows the flowers and leaves of *E. fusca*.



**Figure 1:** Parts of *E. fusca*

Extensive literature research reveals that most of the plant's parts collected from different localities have been evaluated for their diverse biological activities such as cytotoxic, antibacterial, antiviral, anti-inflammatory, antitussive, rheumatism, hematuria, central nervous system depressor, hypotensive, as a uterine stimulant, antiplasmodial, antimalarial, and anti-diarrheal [2, 12-21]. A recent antibacterial study on the plant's aqueous leaf extract against *Porphyromonas gingivalis* gave a stronger bactericidal effect compared to the used standard, chlorhexidine [22]. However, there is no report on the antibacterial activity of the plant on the other bacterial strains that commonly cause infections to humans.

Thus, in this work, *in vitro* antibacterial activity of the leaf extract of *E. fusca* was preliminarily evaluated against several bacterial strains that commonly infect humans which include the Gram-negative *Klebsiella pneumonia*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*,

and the Gram-positive *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus aureus*. In addition, this paper also reports on extract's qualitative phytochemical tests of alkaloids, flavonoids, terpenoids, tannins, and saponins.

## EXPERIMENTAL

### *Plant Materials*

Leaves of *E. fusca* were collected from Bangi, Malaysia in August 2018 and botanically authenticated by a certified botanist, Mr. Ahmad Zainudin Ibrahim. A voucher specimen of the plant (HTBP 5273) was deposited at the Herbarium of Taman Botani, Putrajaya (HTBP). The collected plant materials were cut into small pieces, shade-dried, and then were ground into powder using an electric grinder.

### *Bacterial Strains*

*S. aureus* ATCC 6538 was kindly provided by the Collaborative Drug Discovery Research (CDDR) Laboratory, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), while *K. pneumonia* ATCC 700603, *P. vulgaris* ATCC 6380, *P. aeruginosa* ATCC 10145, *B. subtilis* ATCC 6633, and *B. cereus* ATCC 11778 were acquired from the Microbial Laboratory of Attaur-Rahman Institute for Natural Product Discovery (AuRIns). The obtained bacteria were sub-cultured in Nutrient Broth (NB) and stored at 4 °C until used in experiments.

### *Chemicals and Reagents*

Methanol (MeOH) of analytical grade was purchased from RCI Labscan (Bangkok, Thailand), Bacterial culture media Mueller Hinton Agar (MHA), and Nutrient Broth (NB) were of Oxoid, UK. All other chemicals/reagents were of analytical grade or laboratory grade purchased from reputed manufacturers/companies.

### *Extraction Procedure*

The powdered leaves of *E. fusca* (1.2 kg) were subjected to an exhaustive extraction using MeOH for 72 hours at room temperature. The extract was filtered through Whatman No. 1 filter paper and then was concentrated under reduced pressure by using a rotary evaporator (Buchi, R-215). The concentrated extract was further dried under a fume hood and yielded 60 g of dried crude extract.

The dried crude extract was stored at 4 °C prior to *in vitro* antibacterial and phytochemical analyses.

### ***Antibacterial Assay***

Antibacterial activity of methanolic leaf extract of the plant was carried out against the tested bacterial strains using the agar well diffusion method (WDM). Bacterial culture media MHA was used as a culture medium in the preparation of agar plates while NB was used in the preparation of bacterial inoculum that was adjusted to 0.5 McFarland standard ( $1 \times 10^8$  CFU/mL). All tested bacteria procured from the CDDR and AuRIns' laboratories were sub-cultured in sterilized NB overnight and then were used for the preparation of inoculum.

The McFarland standardized inoculum was prepared using a spectrophotometric method where the optical density (OD) of 0.08 – 0.1 at 600 nm wavelength was adjusted for the bacterial suspension in sterilized NB. An amount of 100  $\mu$ L of the standardized inoculum was seeded on the surface of the agar plate using a sterile L-shaped cell spreader. Then 30  $\mu$ L of the extract solution prepared at a concentration of 100 mg/mL in dimethyl sulfoxide (DMSO) was pipetted into the wells of 6 mm diameter punched in the agar plates using a sterile cork borer. Vancomycin 30  $\mu$ g/well (VAN30) was used as the positive control while 30  $\mu$ L/well of DMSO was used as the negative control. The agar plates were then incubated for 24 hours at 37 °C. The diameter of the zone of inhibition (ZOI) was measured in mm and recorded for both the extract and vancomycin.

### ***Phytochemical Screening***

The qualitative phytochemical analysis of the extract was carried out as per standard protocol [23-24]. Approximately 10 mg of the plant extract was dissolved in 10 ml of methanol and transferred accordingly into five separate test tubes to detect the presence of alkaloids, flavonoids, terpenoids, tannins, and saponins

#### ***Test for alkaloids (Mayer's Reagent)***

Into a test tube containing 2 ml methanolic extract solution, a 0.2 ml dilute hydrochloric acid (HCl) was added, followed by the addition of 1 ml Mayer's reagent. A white precipitate indicates the presence of alkaloids.

### ***Test for flavonoids (Shinoda Test)***

A piece of magnesium ribbon and a few drops of concentrated HCl were added into a test tube containing 2 ml of methanolic extract solution. The presence of flavonoids was recorded by an instant change of the solution colour to red.

### ***Test for terpenoids (Salkowski Test)***

2 ml of chloroform was added into 0.5 ml of the methanolic extract solution in a test tube. This is followed by the addition of 3 mL concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>). The formation of a reddish-brown layer of the interface shows the presence of terpenoids.

### ***Test for tannins (Ferric Chloride Test)***

2 ml of a 5 % iron(III) chloride (FeCl<sub>3</sub>) solution was added into a test tube containing 5 ml of the methanolic extract solution. The formation of a greenish-black precipitate indicates the presence of tannins.

### ***Test for saponins (Froth Test)***

1 ml of methanolic extract solution was further diluted with 5 ml of distilled water and the mixture was vigorously shaken in a closed test tube. The formation of persistent foam shows the presence of saponins.

## **RESULTS AND DISCUSSION**

### ***Antibacterial Activity***

Bacteria, fungus, protozoa, and viruses are the four major types of microorganisms that cause illness [25]. Infections due to these microorganisms have increased dramatically in recent years, and antibiotic resistance has become a growing therapeutic issue [26]. Considering the traditional medicinal role of *E. fusca* in the treatment of several infectious illnesses, the methanolic leaf extract of the plant was evaluated for its antibacterial potential against selected bacterial strains, and the findings are shown in Table 1.

**Table 1:** Data showing ZOI values (mm) of EFMLE and VAN30 against tested bacteria

Bacterial Strain	VAN30 (30 µg/well)	DMSO	EFMLE (3 mg/well)
<sup>a</sup> <i>K. pneumoniae</i> ATCC 700603	NIZ	NIZ	NIZ
<sup>a</sup> <i>P. vulgaris</i> ATCC 6380	16 mm	NIZ	10.5 mm
<sup>a</sup> <i>P. aeruginosa</i> ATCC 10145	NIZ	NIZ	14 mm
<sup>b</sup> <i>B. subtilis</i> ATCC 6633	17 mm	NIZ	12 mm
<sup>b</sup> <i>B. cereus</i> ATCC 11778	17 mm	NIZ	11 mm
<sup>b</sup> <i>S. aureus</i> ATCC 6538	16 mm	NIZ	12 mm

EFMLE: *E. fusca* methanolic leaf extract, NIZ: No inhibition zone, VAN30: Vancomycin 30 µg/disc,

<sup>a</sup>Gram-negative strain, <sup>b</sup>Gram-positive strain

As shown in Table 1, the extract revealed antibacterial effects at the tested concentration of 3 mg/well against all bacterial strains except for *K. pneumoniae* which was also resistant to the standard VAN30. The standard VAN30 showed good enough antibacterial activity on all tested Gram-positive bacteria while it was only active against one of the tested Gram-negative strains that was *P. vulgaris*. However, the resistance of the Gram-negative bacteria to most of the antibiotics is attributed to their second layer of the wall that acts as a barrier against the penetration of antibiotics into the bacterial cell [27]. The plant extract was active against the Gram-negative *P. aeruginosa* with the largest ZOI of 14 mm but interestingly, the standard did not give an inhibition zone. The extract also showed significant activity on the Gram-positive bacterial strains of *B. subtilis* and *S. aureus* with the same ZOI values of 12 mm, while on *P. vulgaris* and *B. cereus* gave moderate ZOI values of 10.5 and 11 mm, respectively. These showed the extract exhibits diverse antibacterial effects on the tested bacterial strains.

Based on the literature, the only reported antibacterial study on *E. fusca* was against the bacterial strains of *Porphyromonas gingivalis* [22]. An aqueous extract of the plant's leaf showed a stronger bactericidal effect compared to the standard used, chlorhexidine. However, several antibacterial potentials on the other species of *Erythrina* were previously reported. These include 1) tests on the bark petroleum ether, chloroform, ethanolic and aqueous extracts of *E. mysorensis* Gamb. against *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis* with benzylpenicillin (100 µg/ml) and streptomycin (100 µg/ml) used as standards [28], 2) on the bark dichloromethane extract of *E. suberosa* (Roxb.) with standard cefixime (no stated concentration) against *S. aureus* exhibited potential antibacterial effect [29], and 3) on the stem bark dichloromethane extract of *E. stricta* Roxb. against methicillin-sensitive *S. aureus* (MSSA) with vancomycin (2 µg/disc) as standard [30].

In general, with substantial supporting literature, *E. fusca* may be considered as a potent antibacterial agent. This study could support the traditional uses of *E. fusca* in treating symptoms related to bacterial infections such as skin itching and inflammations as well as wound infections. The findings here would serve as a basis for further exploration of the antibacterial potential of the plant's leaves. The determination of the minimum inhibitory concentration value of the extract is nevertheless required to further support the findings.

### ***Phytochemical Screening***

The qualitative phytochemical test on methanolic leaf extract of *E. fusca* was performed in order to confirm the presence and absence of phytochemicals such as alkaloids, flavonoids, terpenoids, tannins, and saponins, which might be responsible for the obtained antibacterial effect. Based on phytochemical screening data in Table 2 below, the plant extract contained diverse groups of phytochemicals in which alkaloids, flavonoids, and tannins were present moderately '++'. The terpenoids class of compound was detected in trace amounts, while saponins were absent.

*Erythrina* species have been reported to contain alkaloids and various bioactive flavonoids [31]. However, the most prominent phytochemical constituent of *E. fusca* were alkaloids, where about 12 different alkaloids namely erysodine, erysovine, erysopine, erysotrine, erythraline, erysothiovine, erysothiopine, epierythratidine, erythratinone, erythramine, erythratine and erythrinane have been isolated and identified. A recent ultra-high performance liquid chromatography (UHPLC) analysis on the different parts extract of the plant also supported the presence of alkaloids in *E. fusca* leaf extract [2].

**Table 2:** Detected phytochemical constituents in methanolic leaf extract of *E. fusca*

Phytochemical constituents	Methanolic leaf extract of <i>E. fusca</i>
Alkaloids	++
Flavonoids	++
Terpenoids	+
Tannins	++
Saponins	-

Note: Qualitative approximation scale: '+' trace, '++' moderate, '-' absent

The biological activities executed by any plant are due to the presence of their secondary metabolites. Previous studies have shown that most of the biological activities executed by the *Erythrina* species are due to their alkaloids' constituents. Alkaloidal fraction of *E. crista galli* containing rytharbine, erysotrine, erysotramidine, erysotrine N-oxide and erythratidine, and the newly isolated alkaloid, erythratidine N-oxide, showed a significant inhibitory activity against *P. aeruginosa* [32].

Apart from alkaloids, the other classes of phytochemicals had also been reported to execute antibacterial properties. A study demonstrated that isoflavones and isoflavanones were among the compounds responsible for the antimicrobial activity of *E. stricta* stem bark, with the isolated compounds demonstrating good to moderate activities against the tested bacterial strains of methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), and a multi-drug resistant *S. aureus* (MDRSA), *P. aeruginosa* and *E. coli* [23].

Tannins and saponins were also responsible for the antibacterial effect of *E. variegata* aqueous leaf extract against *E. coli*, *B. cereus*, *P. aeruginosa*, *S. aureus*, and *B. subtilis* [33-34]. These suggest that although alkaloids could be the major group of phytochemicals that are responsible for the antibacterial activity of *E. fusca* leaf extract, however, the other groups of compounds present might also contribute to the effect. Hence, isolation of the active constituents is required to support and further confirm the antibacterial potential of the plant.

## CONCLUSION

The methanolic extract of the leaves of *E. fusca* showed a diverse *in vitro* antibacterial effect against the tested bacterial strains, which the Gram-negative showed more sensitivity compared to the Gram-positive. It was also found that the extract contains different phytoconstituents which include alkaloids, flavonoids, terpenoids, and tannins. Both antibacterial properties and the presence of various phytochemicals in the extract could support the traditional uses of *E. fusca* in treating symptoms related to bacterial infections. The findings would serve as a basis for further exploration of the antibacterial potential of the plant's leaves. However, the determination of the minimum inhibitory concentration value of the extract is required to further support the findings. In-depth phytochemical and antimicrobial studies are also suggested to characterize its potent phytochemicals.





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## AUTHOR'S CONTRIBUTION

Adiez Sapura Azmi and Mohammad Humayoon Amani carried out the research. Adiez Sapura Azmi analyzed the data, wrote, and revised the article. Muhammad Farhan Syakir assisted in the phytochemical works. Fatimah Salim conceptualized the central research idea, provided the theoretical framework, and supervised the research progress. Fatimah Salim and Mohd. Humayoon Amini anchored the review and revisions. All authors read the final manuscript and Fatimah Salim approved its submission.

## CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, and commercial or financial conflicts. The authors declare that there is no conflict of interest.

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