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PROFILING AND IDENTIFICATION OF PHYTOCHEMICALS FROM *ERYTHRINA FUSCA* EXTRACTS AND THEIR ACTIVITY AGAINST SKIN INFECTIOUS BACTERIAL STRAINS THROUGH PROTON NMR-BASED CHEMOMETRIC APPROACH

ADIEZ SAPURA BINTI AZMI

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ABSTRACT

Erythrina fusca (Fabaceae) is a red flowering plant belonging to the *Erythrina* genus. Traditionally, this plant has been claimed to have therapeutic effects on certain symptoms associated with bacterial infections. Although the reported therapeutic activities of the plant are attributed to its alkaloidal content, there is currently no scientific data available to support these claims, particularly regarding the antibacterial activity of the plant against bacterial strains commonly responsible for human infections. Therefore, the objectives of this study were; 1) to extracts alkaloids from *E. fusca*'s leaves using a tartaric and hydrochloric acids through acid-base extraction, 2) to evaluate the antibacterial activity of extracts obtained from different parts of *E. fusca* (leaves, twigs, and flowers) against four human infectious bacterial strains, namely *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Enterobacter cloacae*, and *Enterobacter aerogenes*; 3) to profile and identify the phytochemicals present in the extracts, 4) to determine the variation of phytochemicals between the extracts and the correlation with their antibacterial activity. The antibacterial activity was evaluated using well diffusion method with ciprofloxacin as the positive control. The phytochemicals were profiled and identified using proton nuclear magnetic resonance (^{1}H NMR)-based data analysis, while the phytochemicals variation between different extracts and their correlation with antibacterial activity were determined using principal component analysis (PCA), partial least square discriminant analysis (PLS-DA) and partial least square (PLS) regression. A significantly higher (~12-fold) yield of an alkaloid fraction was obtained using the tartaric acid (983.2 mg) compared to hydrochloric acid (83.5 mg) from 10 g of crude leaf extract. The extracts mostly showed activity against the *S. aureus* with inhibition zones ranging from 6 to 14 mm. A total of 83 phytochemicals were successfully identified mainly from the classes of alkaloids, flavonoids, pterocarpan, terpenes, saponins, and phenols. The PCA showed clear discrimination among the twig and flower extracts, while the PLS-DA discriminated the alkaloidal leaf extract. The PLS analysis showed that the activity against *S. aureus* of the active leaf DCM extract was the pterocarpan dolichin A (**60**), the active twig DCM extract was the alkaloid erysosalvine (**20**), the flavonoid 5,3′-dihydroxy-4′-methoxy-5′-(3-methyl-1,3-butadienyl)-2″,2″-dimethylpyrano-[5,6:6,7]-isoflavanone (**51**), the pterocarpan dolichin A (**60**), the sterols stigmasterol (**72**) and stigmast-3-en-4-one (**73**), the saponins sigmoisides A, B, F, and E (**75**, **76**, **77,** and **78**) and as well as the terpene propyloxyamyrin (**83**), and the active flower hexane extracts was contributed by the flavonoid **51**. The activity on *S. haemolyticus* of the flower hexane and tartaric acid's alkaloidal extracts was correlated to the compound **51**, and collectively contributed by the phytochemicals pterocarpan sandwicensin (**63**) and phenol methyl gallate (**81**), respectively. Furthermore, the activity on *E. cloacae,* and *E. aerogenes* of the flower methanol extract was correlated with and alkaloids erysotrine *N*-oxide (**11**), 10-hydroxy-11-oxoerysotrine (**18**), and magnoflorine (**35**). The present study found that the activity on the tested bacterial strains was collectively contributed by different classes of phytochemicals suggesting the therapeutic reported for the plant could be probably contributed synergically by the above-mentioned phytochemicals. The findings provide evidence to support the plant traditional use and source of antibacterial agents for further studies.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

World Health Organization (WHO) describes herbs as plants as containing compounds that could be used for medicinal purposes (Bakar et al., 2018). Herbs can be been either been consumed directly as a traditional medicine (Ali et al., 2021) or as formulation useful metabolite drugs. The knowledge and uses of traditional medicinal plants by indigenous practitioners and the current drug production are not only useful for maintaining cultural tradition and biodiversity but also for community health care and drug development (Rahman & Parvin, 2015). To date, up to 70% of all clinical available therapies were originally developed from natural products. This mandates the medicinal plants to be the "world's largest pharmacy", opportunities for the researchers to discover new drugs (Jantan, 2004). Health benefits studies medicinal plants have boarded worldwide and captured attention from all researchers around the world including Malaysia (Bakar et al., 2018).

Malaysia is one of the 12 mega diverse countries in the world with the highest plant's endemism including the flowering plants from the genus *Erythrina* (Fabaceae). The genus *Erythrina* is represented by 123 accepted species, mainly distributed in tropical and subtropical regions of the world including South America, South Africa and the Himalayas (Igeh et al., 2022; Kaushal et al., 2020; Rambo et al., 2019a). To the best of author's knowledge, seven species of *Erythrina* are available in Malaysia. The *Erythrina* species are historically used for traditional treatments such as antimalaria, antiseptic, anti-inflammatory, infectious diseases, and promote sleeping and appetite, and (Ahmed et al., 2020; Tjahjandarie et al., 2014). Two major species of the genus are *E. variegata* and *E. fusca*. The former species has been utilized as a sedative and febrifuge, reduce stomach ache and painful menstrual periods (dysmenorrhea), promotes urine production (diuretic), and induce menstruation (Chu et al., 2019; Kumari, P., 2017; de Oliveira et al., 2012). While, *E. fusca* has been used traditionally to relive inflammation, reduce toothache, cleanse putrid ulcers, and cure skin irritation and be eaten as vegetables in Guatemala (Sazed et al., 2020; de Oliveira et al., 2012;