

UNIVERSITI TEKNOLOGI MARA

**PHYTOCHEMICAL ANNOTATION,
IDENTIFICATION, AND ISOLATION
OF *ELEUSINE INDICA* (L.) GAERTN.,
AND ITS ANTIPROLIFERATIVE
AND ANTIBACTERIAL ACTIVITIES**

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Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science
(Applied Chemistry)

Faculty of Applied Science

March 2023

ABSTRACT

Eleusine indica, locally known as ‘rumpu sambau’ is traditionally used to treat various ailments including sprained muscle, asthma, influenza, and pneumonia. Pharmacological investigations revealed that this plant possesses diverse arrays of activities such as antibacterial, cytotoxic, anti-inflammatory, antiplasmodial, and hepatoprotective. Despite many reports on its traditional uses and wide array of biological activities, limited chemical databases are available for this plant. Thus, the aims of this study were to identify and isolate phytochemicals from *E. indica* through conventional (hexane extract), and advanced (methanolic extract) tandem LCMS-based annotation techniques, and to evaluate its antiproliferative and antibacterial activities. The phytochemicals were annotated through tandem LCMS-based analysis using MZmine, GNPS, Compound Discoverer and SIRIUS platforms. Isolation and structural elucidation of phytochemicals were carried out using various chromatographic and spectroscopic techniques, respectively. The antiproliferative activity was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay against human liver cancer (HepG2) and African green monkey kidney epithelial normal (Vero) cell lines, while the antibacterial activity was assessed using disc and agar well diffusion methods against eight bacterial strains namely *Yersinia enterocolitica*, *Enterobacter cloacae*, *Shigella sonnei*, *Salmonella enterica*, *Staphylococcus cohnii*, *Staphylococcus haemolyticus*, *Enterobacter Aerogenes* and *Serratia marcescens* with ciprofloxacin as the positive control. A total of 65 phytochemicals were annotated and identified, while 42 were characterized in methanolic extract. A phytochemical annotated as loliolide (**Me-1**) was subjected to further isolation, purification, and structural elucidation process to verify the reliability on the annotated phytochemicals. The structure of **Me-1** was confirmed to be loliolide (10 mg), thus consequently increase the level of confidence in the other phytochemicals identification. This is the first report on the loliolide isolation from *E. indica*. Phytochemistry work on the hexane extract afforded two compounds elucidated as stigmasterol (8 mg) and β -sitosterol (7 mg). The hexane and methanolic extracts exhibit low activity against both cell lines with the IC_{50} value of 91.02 ± 5.74 and 85.30 ± 3.03 $\mu\text{g/mL}$, but more selective towards cancer cells compared to the normal cells with 10.99 and 7.50 SI values, respectively. The hexane extract showed diverse antibacterial activity, while the methanolic extract was more selective towards the bacteria *Y. enterocolitica subsp. enterocolitica*, *E. aerogenes* and *S. sonnei* with inhibition zone range of 8 – 12 mm.

ACKNOWLEDGEMENT

Alhamdulillah. First of all, I would like to thank Allah, the Most Merciful and the Most Gracious, for giving me the strength and good health to finish up my master project thesis entitled “Phytochemical Annotation, Identification, and Isolation of *Eleusine Indica* (L.) Gaertn., And Its Antiproliferative and Antibacterial Activities”.

This thesis is dedicated to my late father, Mr. Mad Sukor bin Sukaimi and my mother Mrs. Rose Rasinah binti Kadir who have always listened to my problem and give motivation to me to be strong enough to complete this project. Not forgetting to all my four siblings, Nur ‘Izzati, Nur Najihah, Mohd Wafiy and Mohd Nafis, who have giving me their support and love. All of them gave me a lot of advice, guidance, encouragement and help in the financial aids. Without their moral support, I will not be able to do the project on my own.

I would like to express my heartfelt gratitude to my supervisor and my co-supervisor, Dr. Fatimah Salim and Dr. Nurulfazlina Edayah Rasol, for advice, guidance and providing me with a lot of useful information in order to make sure that the laboratory work can be done smoothly. A lot of motivation has been given to me in completing this project. Furthermore, lot of ideas and ways to solve problems especially related to the laboratory work has also been provided. Without their supervision, encouragement and support, this project would not be successfully completed. Along my master journey, I have learned a lot on natural product area’s knowledge and gained useful and specific skills especially related to the instrumentation and software. Besides that, I was trained to be independent, work under pressure and build inner strength to complete this project.

Not forgetting to all staffs and friends, Adiez Sapura, Kak Isna, Kak Wani, Kak tiqa, Kak Fizoh, Kak In, Mizan, Zikry and Farhan from Atta-ur-Rahman Institute for Natural Product Discovery (AuRIns), UiTM Puncak Alam for their kindness and help. Last but not least, millions of thanks to Institute of Research Management and Innovation (IRMI), Universiti Teknologi MARA for funding the study (IRMI grant no. 600-IRMI 5/3/GIP (032/2019)). Their sponsorship and trust give motivation to me to complete this tough journey after facing hard times.

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CHAPTER ONE

INTRODUCTION

1.1 Research Background

Malaysia is rich in plants diversity including flowering plant, ferns, and herbs. It is reported that in Peninsular Malaysia alone has more than 1300 plants which are being used for medicinal purpose (Burkill, 1935). Plants are being used for medicinal purpose due their therapeutic properties executed by the secondary metabolites or also known as phytochemicals presence. Among the plants that are utilised widely for its medicinal values is the *Eleusine indica* (L.) Gaertn. *E. indica* is a perennial herb belongs to the Poaceae family and it can be found in the tropical region South America, Asia, Micronesia, American Samoa and most of the rest of the Pacific Islands from the tropical to subtemperate regions (Lim, 2016).

E. indica is known as Goose grass or locally called “rumput sambau” and it is the only *Eleusine* species that is abundantly found in Malaysia (Lim, 2016). *E. indica* has been used as traditional medicine to treat various diseases around the world including influenza, pneumonia, sprained muscle, hypertension, retention of urine, oliguria and blood coughing (Abdul, Normah, & Khatijah, 2015; De Melo et al., 2005). In Malaysia, the leaf of the plant is pounded to extract its juice traditionally to hasten the delivery of placenta for women and the infusion of the leaves also has been used to help ease vaginal bleeding. In addition, the whole plant can be used to treat inflammatory diseases by using it either dried or fresh. The roots of the plant are useful in treating asthma by drinking decoction of the roots (Abdul et al., 2015). The roots of *E. indica* and *Capsicum* sp. (Solanaceae) were mixed and boiled to treat piles (Kulip et al., 2000). The infusion of the aerial part of *E. indica* with rice also has been used by the local people of KadazanDusun in Sabah to treat symptoms related to flu (Piah, 2017). Cattle, goats, dogs, cats, rats, and chickens consume the young and soft leaves of *E. indica*, which are also used as an antipyretic for herbivores (Morah & Otuk, 2015; Pattanayak & Maity, 2017).

Pharmacological investigations revealed that this plant possess diverse arrays of