UNIVERSITI TEKNOLOGI MARA

ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF ETHYL ACETATE EXTRACT OF TRIGONELLA FOENUM-GRAECUM SEEDS AGAINST URINARY TRACT INFECTION CAUSING BACTERIA AND HUMAN COLORECTAL CANCER CELLS.

NUR HIDAYAH BINTI KAMARUDDIN

Dissertation submitted in partial fulfilment of the requirements for the degree of Bachelor of Pharmacy

Faculty Of Pharmacy

ACKWNOLEDGEMENT

I would like to express my special appreciation and thanks to my main supervisor, Dr. Lim Siong Meng and my co-supervisor, Assoc. Prof. Dr. Kalavathy A/P Ramasamy for their tremendous support and valuable guidance throughout my research study. They have encouraged and inspired me to write consistently. They have also helped me in managing obstacles until the end of this project. Their advised on this research was priceless.

Greatest thanks to postgraduates students of the Collaborative Drug Discovery Research (CDDR) Group for their greatest supports, guidance and advice. I would also like to thank the authority of Universiti Teknologi Mara (UiTM) Puncak Alam Campus, for a conducive environment and wonderful facilities to do research. Besides, I would like to thank the Faculty of Pharmacy for offering the research subject, PHC 567 which has allowed me to explore what research is all about. The research project has served as important exposure to the field of research and development (R&D).

Finally, noble appreciation to my greatest research colleagues Mohd Nadzlen bin Ahamad, Nurul Farhana binti Akan Ajam @ Khaizan, Athirah Aiman binti Sharulnizan and Siti Najihah binti Sa'don for all the wonderful memories, support, assistance and co-operation throughout this study. Deepest appreciation to my beloved families and friends for their understanding and sacrifices. Last but not least, thanks for all prayers that have sustained me.

TABLE OF CONTENT

APPROVAL FORMi
ACKWNOLEDGEMENTii
TABLE OF CONTENTiii
LIST OF TABLESvii
LIST OF FIGURES viii
LIST OF ABBREVIATIONx
ABSTRACTxii
CHAPTER 11
INTRODUCTION1
1.1 Background of study1
1.2 Hypothesis3
1.3 Objectives4
CHAPTER 25
LITERATURE REVIEW5
2.1 Natural plant derived products5
2.1.1 Overview5
2.2 Trigoella foenum-graecum (Fenugreek)
2.2.1 Overview
2.2.2 Plant characteristics
2.2.3 Traditional use9
2.2.4 Chemical constituent of TFG plants10
2.2.5 Scientific evidence of therapeutic properties10

ABSTRACT

The issues of bacterial resistance and chemotherapy-induced side effects had emerged as major obstacles against treatment of urinary tract infections (UTI) and colorectal cancer (CRC). This has prompted the development of new alternative therapeutics from natural sources. The present study investigated the potential of ethyl acetate extracted Trigonella foenum-graecum (TFG) seeds as anti-microbial and cytotoxic agents. Briefly, ethyl acetate extract of TFG seeds was prepared by soaking the TFG seeds in ethyl acetate for 24 h and extracted using rotary evaporator. For assessment of anti-microbial activity, Disk Diffusion Method was adopted by impregnating with 10 µl of extract in different concentration (0.1 mg/ml -100 mg/ml). Gentamicin, ampicillin and penicillin were included as positive controls. The treated bacterial cultures were observed and measured for zone of inhibition after 48 h. As for cytotoxic assay, HCT-116 (2,500 cells/ 180µl) were seeded onto the 96 flat bottom well plate and incubated overnight. Cells were subjected to 72 h treatment with extract ranging (1x10⁻⁵mg/ml - 1x10⁻²mg/ml). Cell viability was evaluate by the SRB assay and data generated was used to plot the dose-response curve from which IC₅₀ was determined. It was found that ethyl acetate extract of TFG seeds was effective against both Gram positive and Gram negative bacteria. Gram positive bacteria were more selective towards this extract. Meanwhile, positive controls were more potent when compared to this extract. On the other hand, this extract inhibited HCT-116 in dose-dependence manner with the yield of IC₅₀=0.026 mg/ml. 5-FU was more potent cytotoxic agent by 43.3 fold. Future study by using difference types of solvent should be conducted to improve this study.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Trigonella foenum-graecum (TFG), alternatively known as fenugreek, is a herbal plant (Gurib-Fakim, 2006) originates from the family of Leguminosea. This plant can be found predominantly in the Middle-East countries (Sindhu, Ratheesh, Shyni, Nambisan & Helen, 2012). It serves as a common diet among the Egyptians. Its seeds, which have a very strong aroma and bitter taste, are used as spice in food preparation (Al-Oqail et al., 2013). The leaves are taken raw as vegetables (Kunnumakkara et al., n.d.). Besides, TFG is also known for its medicinal value. Its therapeutics properties are well documented in the Ayurvedic Medicine System (Khare, 2007). Traditionally, the leaves have been utilised to treat wound infections in poultice (Kunnumakkara et al., n.d.). The leaves can also be taken as tea to promote lactogogue property among lactation mothers. It is useful for easing childbirth during labour. In addition, the leaves can act as soothing agent on skin and hair, anti-ulcer and anti-inflammatory agents (Kunnumakkara et al., n.d.). Apart from the leaves, TFG seeds soaked in water can serve as digestive tonic, demulcent and agent that improves blood circulatory system (Ody, 2000).

Currently, there are many reports on the resistance of bacteria against chemotherapy (Isturiz, 2008), which are reflected in the rise of infectious disease burden (Theuretzbacher, 2013). Bacteria such as *Staphylococcus aureus* (S. aureus),