UNIVERSITI PUTRA MALAYSIA

ISOLATION, IDENTIFICATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF THE SEMI-PURIFIED COMPOUND FROM STROBILANTHES CRISPUS (L. BREMEK)

AHMED FARESS HAMAD ABOU MUAMAR

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By

AHMED FARESS HAMAD ABOU MUAMAR

Thesis Submitted in Fulfillment of the Requirements for the Degree of Master of Science in the Faculty of Medicine and Health Science University Putra Malaysia

December 1999

DEDICATION

This research project is dedicated to my family, father,

mother,

brothers, uncle, sister and to my special friend Karim.

It's difficult to say what is impossible, for the dream of yesterday is the hope of today and reality of tomorrow

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Chairman: **Professor Dr. Abdul Salam Abdullah**

Faculty: **Medicine and Health Science**

This study involved isolation, purification and identification of bioactive compound from the leaves of Kecibiling, *Strobilanthes crispus.* The bioactive compound obtained was tested for its antibacterial activity both *in vitro* and *in vivo.*

Chemical investigation on the leaves using methanol, Column Chromatography and liquid- liquid extraction of the oily fraction resulted in the isolation, purification and identification of the active compound, verbascoside.

The structure was determined using modern spectroscopic techniques such as UV, IR, ^{1}H-NMR, ^{13}C-NMR and by comparison with the literature.

The bioassay of the activities of the crude extract of the leaves of different solvents and butanol fractions was performed against both gram positive and gram negative bacteria such as *Staphylococcus aureus*, *Streptococcus faecalis*, *Vibrio cholerae* and *Pseudomonas aeruginosa* according to the procedure described in the literature. These tests showed that the extracts and the fractions were effective against the two strains of bacteria.

The *in vitro* antibacterial activity of the compound was tested against three types of bacteria, i:e *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa and* compared with other drugs like Penicillin (10 µg/disc), Erythromycin (15 µg/disc), and Tetracycline (30 µg/ml). The results showed that the compound was very effective as an antibacterial agent. *In vivo* testing also showed good effect against *Staphylococcus aureus* and *Salmonella typhi* and it's effective dose against both organisms was calculated to be (38. 481 mg / kg) and (35.539 mg / kg) respectively.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan ijazah Master Sains

PENGASINGAN, PENGENALAN DAN BIOAKTIVITI PRODUK SEMULAJADI DARIPADA *Strobilanthes crispus* (L. Bremek)

Oleh

AHMED FARESS HAMAD ABOU MUAMAR

Disember 1999

Pengerusi: Profesor Dr. Abdul Salam Abdullah

Fakulti: Perubatan dan Sains Kesihatan

Dalam kajian ini, sebatian bioaktif *Strobilanthes crispus*, telah diasingkan dan dikenalpasti. Daun tumbuhan ini diekstrak dengan metanol, dan seterusnya dipisahkan seterusnya melalui turus kromatografi dan diikuti dengan ekstrak cecair-cecair bahagian berminyak, menghasilkan pengasingan sebatian aktif yang dikenali sebagai verbaskosida.

Struktur sebatian ini telah ditentukan menggunakan teknik spektroskopi moder seperti UV, IR, H-NMR, C-NMR dan secara perbandingan dengan kajian terdahulu.

Aktiviti-aktiviti bioasai ekstrak kasar menggunakan pelarut-pelarut berbeza dan juga fraksi butanol daun *S. crispus* telah dijalankan ke atas kedua-dua bakteria gram positif dan gram negatif berpandukan prosedur yang diterangkan dalam kajian terdahulu. Ujian-ujian ini menunjukkan kesan positif ekstrak-ekstrak dan juga fraksi-fraksi ke atas dua strain bakteria ini.

Aktiviti antibakteria verbaskosida telah diuji secara *in vitro* ke atas tiga bakteria iaitu *Staphylococcus aureus, Salmonella typhi* dan *Pseudomonas aeruginosa* dan dibandingkan dengan drug-drug lain, iaitu Penicillin (10 μg/cakera), Erythromycin (15 μg/cakera) dan Tetracycline (30 mg/ml). Hasilnya menunjukkan sebatian ini sungguh efektif sebagai agen antibakteria. Ujian *in vivo* juga memberikan keputusan yang efektif ke atas *Staphylococcus aureus* dan *Salmonella typhi*. Dos efektif ke atas kedua-duanya juga telah dikira, dan adalah 38.481 mg/kg dan 35.539 mg/kg masing-masing.

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I would like to express my most sincere and warmest gratitude to my father, mother, brothers, sister, relatives and to the person I love for their prayers, love, generosity and moral inputs during my study.

Special thanks go to the brothers in Arab Student Aid International (ASAI) for their help and support. I also would like to convey my special thanks to my uncle Ibrahim for his support. I am grateful to my brother Mohamad Sharif for his kindness and moral support.

I certify that an Examination Committee met on December 24, 1999, to conduct the final examination of Ahmed Faress Hamad Abou Muamar , on his Master of Science thesis entitled "Isolation, Identification and Evaluation of Antibacterial Activity of the Semi-Purified Compound from *Strobilanthes crispus* (L.Bremek) in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and was accepted as fulfillment of the requirements for the degree of Master of Science.

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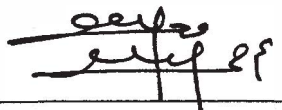
Date: 10 FEB 2000

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that this thesis has not been previously on concurrently submitted for any other degree at UPM or any other institutions.

Signed



(Ahmed Faress Hamad Abou Muamar)

Date: January 19, 2000

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LIST OF ABBREVIATIONS

UV	Ultraviolet
br	Broad
sm	Small
sh	Sharp
md	Medium
dd	Doublet of doublet
NMR	Nuclear Magnetic Resonance
H	Hydrogen
C	Carbon
IR	Infrared
m	Multiplet
t	Triplet
s	Single
MIC	Minimum Inhibition Concentration
MLD	Minimum Lethal Dose
w	Week

CHAPTER ONE

INTRODUCTION

Strobilanthes crispus (L.) Bremek *Saricocolyx crispus* (L.) Bremek (Acanthacia) plant is native to countries from Madagoskar, which is commonly known as daun picah beling in Indonesia and enyoh kelo, kecibeling or kejibeling in Jawa (Suharto, 1977).

This bush-like plant can be found on riverbank or abandoned field while some Javanese use this plant as fence.

The leaves are oblong-lanceolate, rather obtuse and shallowly crenate-crispate (Apoteker, 1977). The upper surface of the leaves are darker green in color and less rough than the other side (Suharto, 1977). The leaves are very scabrid on both surfaces and covered with short hairs. The flowers are short, dense and panicled spikes (Apoteker, 1977). The plant can be easily replanted by using the stacks (Heyne, 1987).

Even though there are very little record of this plant being used for medicinal purposes, it has been found by a study in Indonesia that an infusion of the dried leaves of *Strobilanthes crispus* has been used as antidiabetic, diuretic, antilithic and laxative. The plant has many cystoliths calcium carbonate and an infusion of this plant leaves is mildly alkaline (Perry & Metzger, 1980) which give slightly bitter taste (Suharto, 1977).

Suharto (1977) found that the leaves contain 10-13% moisture on wet basis, not more than 16% acid soluble ash, less than 4% water soluble fiber, more than 16% ethanol soluble fiber, and less than 2% of foreign organic matters. It was also reported that the leaves has a high potassium silicate (Suharto, 1977) or potassium and silicic acid (Perry & Metzger, 1980), and some chemical extracts like caffiec acid, glycosidic ester, verbascoside and phenolic acid (Soediro *et al.*, 1983).

A recent study indicate that the water extract of *Strobilanthes crispus* contains compouds with very high binding affinity to protein molecules and bind to the active site of rverse transcriptase lead to inhibit the proliferation of retroviruses (an agent in viral diseases such as AIDS and adult T-cell leukemia) (Kusumoto et al., 1992)