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

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

ISOLATION, IDENTIFICATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF THE SEMI-PURIFIED COMPOUND FROM Strobilanthes crispus (L. Bremek)

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December 1999

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, \textsuperscript{1}H-NMR, \textsuperscript{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 \textit{Staphylococcus aureus}, \textit{Streptococcus faecalis}, \textit{Vibrio cholerae} and \textit{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 \textit{in vitro} antibacterial activity of the compound was tested against three types of bacteria, i.e \textit{Staphylococcus aureus}, \textit{Salmonella typhi} and \textit{Pseudomonas aeruginosa} and compared with other drugs like Penicillin (10 $\mu$g/disc), Erythromycin (15 $\mu$g/disc), and Tetracycline (30 $\mu$g/ml). The results showed that the compound was very effective as an antibacterial agent. \textit{In vivo} testing also showed good effect against \textit{Staphylococcus aureus} and \textit{Salmonella typhi} and its 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 ceceir-ceceir bahagiañ 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 \( \mu g/\)cakera), Erythromycin (15 \( \mu g/\)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.
ACKNOWLEDGEMENTS

In the name of Allah, the most benevolent and most merciful. I would like to thank my chairman of supervisory committee, Professor Dr Abdul Salam Abdullah whose expert guidance, advice and support has helped me to complete this research. His kindness, affection, encouragement and moral support gave me the courage and ability to overcome all the problems, which I faced from time to time during the course of my work. I would like to extend my heartfelt appreciation to him for his invaluable advice and continuous comments, which brighten my future through the experiences that I gained from him.

I am also indebted to members of my supervisory committee, and I wish to express my special appreciation to Professor Dr Nordin Hj. Lajis for his interest, suggestions, and help in the isolation and identification of the compound throughout my research. A word of thanks and very sincere gratitude and appreciation to my co-supervisor Associate Professor Dr Maznah Ismail, Head of Department of Nutrition and Health Sciences for providing me all the facilities to carry out the research work.
I am also very grateful to Dr Mariana Nor Shamsudin for her concern and continuous suggestions in the bioassay tests. I wish to extend my warmest thanks particularly to Dr. Hatim Ali Elsheikh for the time, effort and continuous encouragement and suggestions he has kindly provided in calculating the effective dose of the drug.

I also appreciate the assistance given to me by all the staff of the Department of Nutrition and Health Sciences and Biomedical Sciences, especially Siti Muskinah Hj Mansor and Mr Zainan for their kindness and cooperation. I am grateful to my brother Dr Khairi for his kindness and moral support.

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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.

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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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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

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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 panicked 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 caffeic acid, glycosidic ester, verbascoside and phenolic acid (Soediro et al., 1983).

A recent study indicate that the water extract of *Strobilanthes crispus* contains compounds with very high binding affinity to protein molecules and bind to the active site of reverse 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)