

UNIVERSITI TEKNOLOGI MARA

**ANTITUMOUR ACTIVITY OF SRJ13, A NEW
DERIVATIVE OF ANDROGRAPHOLIDE,
AGAINST BREAST CANCER CELL LINES**

NOR HAFIZAH BINTI HASSAN

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

October 2005

ACKNOWLEDGEMENTS

I am very grateful and thankful to Almighty Allah S.W.T in giving me patience and strength to complete this project. In the process of accomplishing this task, I was fortunate enough to work with people who had contributed greatly to the success of this research project.

Firstly, I would like to take this opportunity to express my gratitude and appreciation to my supervisor, Mr Lim Siong Meng for his guidance, invaluable advises and courage, constructive comments and patience during the course of this project.

Next, I would like to express my appreciation to the Cancer Research and Drug Discovery Group at the Department of Biomedical Sciences, Universiti Putra Malaysia (UPM). Many thanks to my co-supervisor; Dr. Johnson Stanslas, for allowing me to use the facilities of the laboratory. His opinions and guidance are very much appreciated. Besides, I would also like to express my heartfelt thanks to Mr Lim Siang Hui who had really taught and guided me regarding the cell culture techniques which is a total new experience for me. Not to forget Miss Audrey Yong, Miss Sandra a/p Maniam and Mr Tang Seng Chuan for all their supports, advises and opinions.

Many thanks also to Dr. Kalavathy for her guidance on the write-up of my thesis. I would like to express my appreciation to all my colleagues for their comments and opinions. Last but not least, special thanks to my family members for all their love, patience and willingness to fetch me each time I need to travel down to UPM.

TABLE OF CONTENTS

	Page
TITLE PAGE	
APPROVAL FORM	
ACKNOWLEDGEMENT	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF ABBREVIATION	ix
ABSTRACT	x
(CHAPTER ONE) INTRODUCTION	1
(CHAPTER TWO) LITERATURE REVIEW	4
2.1 Cancer	4
2.2 Breast cancer	5
2.2.1 Classification and staging of breast cancer	7
2.3 Treatment of breast cancer	9
2.3.1 Tamoxifen	9
2.3.2 Paclitaxel	10
2.3.3 Multidrug resistant	11
2.4 <i>Andrographis paniculata</i>	12
2.4.1 SRJ13	13
(CHAPTER THREE) MATERIALS AND METHODS	14
3.1 Materials	14
3.1.1 Reagents	14
3.1.2 Breast cancer cell lines	14
3.1.3 Drugs	16
3.1.4 Instruments	16

ABSTRACT

Breast cancer is the most common cancer among women. Chemotherapy is not selective in its therapeutic effects. Hence, the occurrence of many side effects. The tendency of breast cancer developing resistance towards standard anticancer agent is also a great hurdle yet to be overcome. Thus, the discovery of a novel anticancer agent is vital. SRJ13 is a semisynthetic compound, derived from andrographolide. Previous studies revealed that andrographolide possess antitumor activity against various types of human cancers including breast cancers. Therefore, SRJ13 might exhibit better antitumour effect. This study aimed to investigate the antitumor activity of SRJ13 against different breast cancer cell lines. MCF7 and T47D are both hormone-dependent cell lines whereas MDA-MB-231 and MDA-MB-468 are both hormone-independent. Another objective of this study is to compare the anticancer activity of SRJ13 against these breast cancer cell lines to that of the andrographolide and tamoxifen. The potential synergistic interaction between SRJ13 and tamoxifen against MCF7 and MDA-MB-468 would also be investigated. The cell cultures were sustained by RPMI 1640, supplemented with 10% FBS and 1% penicillin-streptomycin. The breast cancer cells were then treated singly with the respective agents at different concentrations, ranging from 0.1 μ M to 100 μ M. The cells were incubated for 96 hours after which viability of the cells was determined using the MTT assay. Data generated from the microplate reader was used to plot the dose response curve. As for the concomitant treatment of SRJ13 and tamoxifen, the procedures stated above apply. The concentration range of both agents was between 2 times to 0.2 times of their respective GI₅₀. Data obtained were then analysed using the median effect analysis. Findings of this study showed that SRJ13 had reduced the growth of all the breast cancer cell lines. SRJ13 is most effective against MDA-MB-468. When compared the anticancer activity of SRJ13 to that of the andrographolide, results showed that andrographolide is more potent against all the breast cancer cell lines except for MCF7. However, further statistical analysis revealed that the differences observed between SRJ13 and tamoxifen are insignificant. Interestingly, SRJ13 had been found to be a better choice of anticancer agent compare to tamoxifen when treated against MDA-MB-231 and MDA-MB-468 ($p < 0.05$). As for combination treatment of SRJ13 and tamoxifen against MDA-MB-468, the overall inhibiting effects were found statistically insignificant. However, synergistic effect of the combination treatment was observed at two times GI₅₀ of the respective drugs which treated against MDA-MB-468. The potential of SRJ13 being used in combination with other standard agents should be investigated more extensively. Further studies should also be conducted to uncover its mechanism of action and pharmacokinetic profile.

CHAPTER 1

INTRODUCTION

Cancer is the uncontrolled growth of abnormal cells. Metastasis of abnormal cells may affect almost any tissue of the biological system. It causes 7 million deaths per year, which comprises of 12.5% of deaths worldwide (WHO, 2004). Meanwhile, the World Cancer Report (2002) revealed that the global cancer rates could increase by 50% to 15 million at 2020 and this in turn will cause 10 million new deaths. The Ministry of Health Malaysia in their recent Health Facts 2002 report had stated that cancers were the third most common disease that had caused deaths in Malaysia government hospitals in both the years of 2002 and 2003 (MOH, 2004). Among various types of tumour cancers, breast cancer is the most common cancer affecting women. Statistic done in year 1998 had shown that breast cancer is the third leading cancer to cause deaths among the Malaysian women (cited by Lim, 2002).

Cancerous cells such as that of the breast cancers occur when normal cells undergo mutation of the DNA either through genetics hereditary or acquisition. There are three main approaches to breast cancer treatment, which include surgery, radiotherapy and chemotherapy. For the treatment of breast cancer with local invasiveness, surgical excision and irradiation are the two modalities of choices. While at late stage whereby the cancerous cells have metastasized to adjacent and distant