UNIVERSITI TEKNOLOGI MARA

ELUCIDATION OF APOPTOTIC PATHWAY ON HUMAN BREAST ADENOCARCINOMA CELL LINES MEDIATED BY *LABISIA PUMILA VAR. ALATA* EXTRACT

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ABSTRACT

Labisia pumila, or locally known as Kacip Fatimah were widely used by women in Malaysia to treat post-partum illnesses. Due to the increase uses of complementary medicine in breast cancer patient, this herbaceous shrub has been exploited as a replacement to the conventional therapies. Many studies indicated that *Labisia pumila* exerts a wide range of biological activities, including the anti-proliferation effect. However, details of the mechanism were still poorly understood. Therefore, this study was conducted to elucidate the mechanism of *Labisia pumila var. alata* (*Lpva*) inducing anti-proliferative effect towards breast adenocarcinoma (MCF-7) cell lines and the underlying mechanism. The treated cells were subjected to viability assay (MTT assay), apoptosis assay (Flow cytometry), and protein expression (Western blot). Moreover, *Lpva* was also tested for its estrogenic activity (Molecular docking and estrogen binding assay). Aqueous extracts from *Lpva* showed anti-proliferative activities in a dose- and time-dependent manner (p>0.005). Flow cytometry analysis showed that the anti-proliferative effect of *Lpva* induced through the apoptosis pathway where the early apoptotic cell population increase from 20 % in 24 hours to 50% in 72 hours. Molecular docking simulation showed that *Lpva* phytochemical able to bind to estrogen receptors with diadzein gives the lowest affinity at -9.1 for both estrogen receptors alpha and beta, while estrogen binding assay proved that aqueous extract of *Lpva* able to bind to estrogen receptor alpha and estrogen receptor beta. Furthermore, estrogen antagonist, fulvestrant (ICI 182,780), proved that *Lpva* aqueous extract exerts their anti-proliferative effect through the estrogen receptors of MCF-7 cells. Western blot analysis showed that *Lpva* extracts induced apoptosis through both intrinsic and extrinsic pathways. *Lpva* significantly increase the expression of caspase 8 and 9 at IC_{50} and IC_{75} concentration with caspase 9 showed the highest expression (1.47 protein ratio at IC_{50} and 1.57 protein ratio at IC_{75}) (p>0.01). Western blot analysis also suggested that *Lpva* down-regulated the expression of Bcl-2 at IC_{75} (0.38 protein ratio) and up-regulated the expression of pro-apoptotic protein Bax at IC_{50} and IC_{75} (0.64 and 1.14 protein ratio) (p>0.01). These results elucidate the mechanism of *Lpva* anti-proliferative effect on MCF-7 cells.

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TABLE OF CONTENTS

		Page
CONFIRMATION BY PANEL OF EXAMINERS		**ii**
AUTHOR'S DECLARATION		**iii**
ABSTRACT		**iv**
ACKNOWLEDGEMENT		**v**
TABLE OF CONTENTS		**vi**
LIST OF TABLES		**x**
LIST OF FIGURES		**xi**
LIST OF SYMBOLS		**xiii**
LIST OF ABBREVIATIONS		**xiv**
CHAPTER ONE: INTRODUCTIONS		**1**
1.1	Research Background	1
1.2	Problem Statement	2
1.3	Research Objectives	3
1.4	Significance of Study	3
1.5	Scope of Study	3
1.6	Limitation of Study	4
CHAPTER TWO: LITERATURE REVIEWS		**5**
2.1	Apoptosis	5
2.1.1	Morphological Characteristic of Apoptosis	5
2.1.2	Apoptosis Pathways	7
	2.1.2.1 Extrinsic Pathway or Death Receptor-Mediated Apoptosis Pathway	8
	2.1.2.2 Intrinsic Pathway or Mitochondrial Apoptosis Pathway	8
	2.1.2.3 Apoptosis Execution Pathway	10
2.1.3	Bcl-2 Family Proteins	10
2.1.4	Role of Apoptosis in Cancer Cell	12

CHAPTER ONE
INTRODUCTION

1.1 Research Background

Apoptosis is a genetically directed process of cell destruction which is marked by a fragmentation of nuclear DNA and the presence of apoptotic bodies. It is a normal physiological process to eliminate age, damaged, or unwanted cells. These processes might be interrupted by gene mutation and result in uncontrolled cell growth and tumor formation.

In human cells, the apoptosis process triggered by a member of the Fas and TNF receptor family. Apoptosis often involving in the destruction of mitochondrial membrane integrity which in turn release cytochrome c into the cytosol and this process serve as a decisive factor for the onset of cell death (Marks et al., 2009). Few other cellular mechanisms have been identified to be involved in the process of apoptosis, including the regulation of biochemical activities of caspases (Marks et al., 2009). When these caspases are activated, it can specifically cleave cellular death substrate that leads to morphological and biochemical changes of apoptosis process (Portt et al., 2011).

Breast cancer, amongst all types of cancer is one of the most challenging disease and responsible for large number of cancer related death. Chemotherapy, radiotherapy, hormonal therapy and surgery have been used as treatment for a long time. Due to its severe side effects and multidrug resistance, many breast cancer patients changing from conventional to complementary and alternative treatment (Mitra & Dash, 2018).

Nowadays, studies across the globe have advanced in understanding the mechanism of various natural anticancer agents indicate that whether the diverse chemical nature of the anticancer drug, most of them stimulate apoptosis in most test tumor cells (Chen & Chien, 2014; Kang et al., 2009). Few examples like daidzein, a potent chemopreventive agent, found in many types of plant can induce apoptosis in cancer cells (Jin et al., 2010). Vernodalin isolated from *Centratherum anthelminticum* (L.) seeds has been validated to induce apoptosis in breast cancer cell via a