

CHAPTER I

INTRODUCTION

1. Rationale and Background

Morus alba L. (white mulberry) is a woody perennial tree belonging to the Moraceae family. Root bark of *M. alba* (sang bai pi or *Cortex mori*) was used as traditional medicine for anti-tussive, anti-inflammation and anti-asthmatic (Piao et al., 2010, cited from Chinese Pharmacopoeia Commission, 2005). The root bark contains several bioactive metabolites including stilbenes, flavonoids, benzofurans and coumarins. Mulberroside A (MuA) is a major stilbene glycoside that has been identified as the active compound from root bark of mulberry (Piao et al., 2010). The MuA was reported antioxidant, anti-tyrosinase activities and also used as bioactive material in cosmetic industry. It can be converted by intestine microbial or directly by glycosidase to aglycone part, oxyresveratrol (Kim et al., 2010; Mei et al., 2012). Oxyresveratrol shows many pharmacological effects including anti-tyrosinase activity (Tengamnuay et al., 2006), anti-herpes simplex (Lipipun et al., 2011) and anti-African swine fever virus (Galindo et al., 2011), antioxidant activity (Aftab et al., 2010), hepatoprotective effect (Oh et al., 2002) and neuroprotective effect (Horn et al., 2004).

Tissue culture technique was developed for rapid propagation on the large scale cultures of *M. alba* cultivation (Anis et al., 2003). In general, culture conditions including nutrition, environment protection and temperature are controllable by tissue culture method. In addition, cultured tissue can grow in a laboratory and accumulate high amount of chemicals which founded in a parent plant with short growth cycles (Rao, Ravishankar, 2002). Therefore, this method is also an interesting tool for the secondary metabolites production from *in vitro* culture of *M. alba*.

One of the effective strategies used for the enhancement of secondary metabolites accumulations is to stimulate plant defensive response by elicitation. Salicylic acid, methyl jasmonate, yeast extract, chitosan and fungal extract were reported as stilbenes

elicitors (Aziz et al., 2006; Belhadj et al., 2006; Roat, Ramawat, 2009). Besides, endophytes and common microbes isolated from their host plant have been reported for elicitation effect on production of their host bioactive metabolites (Chong et al., 2009; Orlita et al., 2007).

Several methods were developed to determine stilbenoids including high-performance liquid chromatography (HPLC) (Piao et al., 2010), gas chromatography-mass spectrometry (GC-MS) (Potter et al., 2002) and thin layer chromatography (TLC) densitometry (Maneechai et al., 2009). Determination of the MuA has been performed by HPLC method (Piao et al., 2010).

Enzyme-linked immunosorbent assay (ELISA) was reported to be simple, specific, rapid and high sensitivity method to investigate levels of plant secondary metabolites such as isoflavonoids (Pongkitwitoon et al., 2010), aculeatiside A (Putalun et al., 2002) and asiaticoside (Tassanawat et al., 2012). To the best of our knowledge, no antibody against MuA was reported.

In the present study, we aim to produce polyclonal antibody against MuA for MuA determination in various samples by ELISA method. Additionally, we also aim at investigate the effects of elicitors on the cell and root cultures of *M. alba* as well as the evaluation of MuA production, α -glucosidase and tyrosinase inhibitory from the *in vitro* cultures and microbes isolated from *M. alba*.

2. Objectives of the Study

- 2.1 To produce and characterize polyclonal antibody against MuA.
- 2.2 To establish cell and root cultures of *M. alba*.
- 2.3 To study effect of elicitors on the cell and root cultures of *M. alba*.
- 2.4 To evaluate MuA production, α -glucosidase and tyrosinase inhibitory effects from the *in vitro* cultures and microbes isolated from *M. alba*.

3. Scope and Limitation of the Study

3.1 Determination of MuA by indirect competitive ELISA using specific MuA polyclonal antibody.

3.2 Induction of cell suspension and root cultures from *M. alba*.

3.3 Enhancement of MuA production in the cell suspension and root cultures by elicitation with yeast, methyl jasmonate, chitosan, salicylic acid and the extracts of microbial isolated from *M. alba*.

3.4 Evaluation of α -glucosidase and tyrosinase inhibitory in *in vitro* cultures and microbes isolated from *M. alba*.

4. Places for Study

Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. Northeast Laboratory Animal Center, Khon Kaen University, Thailand and Graduate School of Pharmaceutical Sciences, Kyushu University, Japan.