CHAPTER 1

INTRODUCTION

Artemisinin or qinghaosu is a sesquiterpene lactone endoperoxide compound. It is isolated from *Artemisinin annua* L. and has been used as Chinese herbal remedy for at least 2,000 years (Zhan *et al*., 2002b). Artemisinin is an anti-malarial agent that is effective against multidrug-resistant *Plasmodium falciparum* without any reported cases of resistance (Krishna *et al*., 2008). In addition, artemisinin and its derivatives, artesunate, artemether, arteether and dihydroartemisinin display anticancer activity by inducing apoptosis (Meshnick, 2002; Meshnick *et al*., 1991; Nakase *et al*., 2008; O'Neill *et al*., 2010; Timothy J, 2008). However, the biological activity of artemisinin is limited due to its short elimination half-life, toxicity, and water solubility (Krishna *et al*., 2008; Meshnick, 2002; Rydén and Kayser, 2007). The quantitative structure-activity relationship (QSAR) studies suggested that the structural modification of artemisinin with steric bulk at 7β-position of artemisinin may offer better antimalarial activity and water solubility (Avery *et al*., 1999). The structural modifications of artemisinin by chemical and biological approaches were investigated in order to improve its biological activity. Ekthawatchai *et al.* (2001) reported a successful chemical transformation of artemisitene into artemisinin monomers, dimers, trimers, and tetramers. The majority of the C-16 artemisinin derivatives either showed higher antimalarial activity or comparable activity to that of artemisinin with low cytotoxicity against human epidermoid carcinoma (KB), human breast cancer (BC),

and African green monkey kidney fibroblast (Vero cells). The antimalarial activities of artemisinin dimers, trimers and tetramers were also greater than artemisinin but showed high cytotoxicity against KB, BC, and Vero cell lines (Ekthawatchai *et al*., 2001). Another structural modification approach is biotransformation which is frequently used for the generation of new or novel bioactive products or improvement of the biological activities of bioactive products (Loughlin, 2000). Several successful biotransformations of artemisinin and its derivatives by fungi were reported. Lee *et al.* (1989) discovered that *Nocardia corallina* ATCC 19070 and *Penicillium chrysogenum* ATCC 9480 were able to transform artemisinin to deoxyartemisinin and 3α-hydroxydeoxyartemisinin (Lee *et al*., 1989). Zhan and colleagues (2002) also found that *Mucor polymorphosporus* was able to convert artemisinin into 9β-hydroxyartemisinin, 3β-hydroxyartemisinin, deoxyartemisinin and 3β-hydroxydeoxyartemisinin (Zhan *et al*., 2002a). Another similar finding was reported by Parshikov *et al*. (2006); artemisinin was transformed to 5β-hydroxyartemisinin and 7β-hydroxyartemisinin by *Eurotium amstelodami* and *Aspergillus niger* (Parshikov *et al*., 2006). Additionally, Srivastava and co-workers (2009) reported that deoxyartemisinin, which was a transformed product of artemisinin by *Aspergillus flavus*, showed higher antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus mutans* than that of artemisinin (Srivastava *et al*., 2009). Furthermore, Goswami *et al.* (2010) found that artemisinin was transformed to 9β-acetoxy artemisinin and 9α-hydroxyartemisinin by *Penicillium simplissimum*. The 9β-acetoxy artemisinin showed particularly inhibitory to colon HCT-15, lung A549 and neuroblastoma IMR-32 cell lines (Goswami *et al*., 2010). According to these successful artemisinin transformation by fungi, in this study, five

strains of *Aspergillus* sp. which were *A. oryzae* (Ozykat-1), *A. niger* TISTR 3254, *A. usamii* TISTR 3258, *A. terricola* TISTR 3109 and *A. melleus* TISTR 3128 were used to assess the abilities to transform artemisinin. Some potential strains were selected to find a suitable condition to transform artemisinin. Then, the structure of the transformed product was identified by using various spectroscopic techniques. Afterward, the *in vitro* cytotoxicity, antimalarial and antimicrobial activities of purified transformed product were investigated in order to evaluate any effect of modified-artemisinin on biological activities.

The objectives of this study were as follows:

1. To screen for potential strains of *Aspergillus* sp. in the transformation of artemisinin
2. To find suitable transformation conditions and identify fungal transformed products
3. To evaluate biological activities of the transformed products