CHAPTER I

INTRODUCTION

Cancer is a major public problem world widely. This disease significantly increases in morbidity and mortality rate. In Thailand, it has been the leading cause of death for several years, with an increase in the death rate every year (National statistical office of Thailand, 2003). The Ministry of Public Health of Thailand reported that cancer killed more than 60,000 Thai people per year. The disease affects men and women alike. Lung cancer and cervix cancer are the most cancer found in men and women respectively (The Ministry of Public Health of Thailand, The Information and Public Relations Office, 2008).

Thai medicinal plants are a valuable source of novel anticancer agents. Many Thai people use traditional herbs as an alternative treatment for cancer (Subchareon, 1998; Itharat and Ooraikul, 2007). In spite of many publications demonstrated for cytotoxicity of Thai traditional plants against cancer cell lines but the specific mechanisms had not been established. Here, we pointed out the specific mechanism, Topoisomerase I inhibitory activity, of cytotoxic agents from plant extracts. Plants selected for the screen was based largely on the knowledge of Thai folk medicine and on publications showing cytotoxicity against cancer cell lines.

The DNA topoisomerases are essential for DNA replication, transcription, recombination, as well as for chromosome compaction and segregation (Pommier, 1998). Type I topoisomerase (top 1), is a ubiquitous enzyme, which alters DNA topology by reversible cleavage of DNA and by formation of a transient phosphodiester-tyrosine linkage, the cleavable complex. A tyrosyl group of the enzyme attacks a phosphodiester bond on DNA and remains covalently attached to one side of the break while releasing a free hydroxylated strand (Wang, 1996; Forterre *et al.*, 2007) (Figure 1.1). Trapping of the cleavable complex could be poison into the cells. The collision of advancing replication forks with compound-stabilized intermediates appears to produce the cytotoxic DNA lesions that signal cell cycle arrest and cause cell death (Pommier *et al.*, 1998; Strumberg *et al.*,

2000). Therefore, inhibitory of topoisomerase I activity was a great harm to a cellular genome to develop a nuclear toxin that can efficiently kill cancer cells (Beretta, 1999). As a consequence, this enzyme is potential target for cytotoxic drugs.

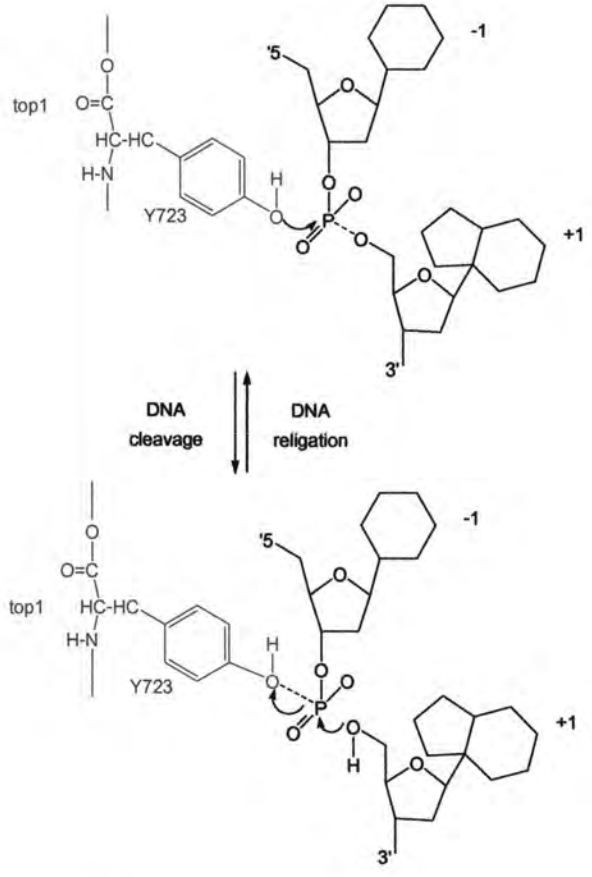

Figure 1.1 DNA topoisomerase I-mediated DNA cleavage and religation. Y723 refers to the tyrosine involved in the trans-esterification reaction with the DNA. By convention, the bases flanking the top1 cleavage site are referred to as -1 and +1 for the bases at the 3' and 5' DNA termini, respectively (modified from Pommier, 1998).

The study of bioactive compounds from plants has required the development of bioassay techniques, especially yeast model which allow a large number of plant extracts to be screened for their activities. The budding yeast *Saccharomyces cerevisiae* has been a valuable model in establishing eukaryotic DNA topoisomerase I as the cellular target of

specific antineoplastic agent including camptothecin, aclacinomycin A, and R-3, a rebeccamycin analogue (Osheroff and Bjornsti, 2001). This study started from *topoisomerase* I deletion mutant yeast (*S. cerevisiae* RS190) strain, then generated the new transformant containing *Arabidopsis thaliana topoisomerase* I gene to achieve inducible or constitutive expression of the *topoisomerase I* from *A. thaliana*. For yeast cell-based assay, in the presence of the topoisomerase I–specific poison compounds from extracts, the rejoining step was showed significantly, resulting in *topoisomerase* I being trapped in a covalent complex with DNA. Suicide was created in transformant yeast because replication forks collide with the compound-enzyme-DNA complex to produce double-stranded DNA break. Compounds exhibiting topoisomerase I inhibitory activity can be identified (Bjornsti *et al.*, 1989; Eng *et al.*, 1988; Kieber, Tissier, and Signer, 1992; Reid *et al.*, 1998). This model system has useful for the assay and discovery of novel topoisomerase I-targeted agents from Thai medicinal plants.

In this study, 27 medicinal plants were screened to determine their ability to inhibit Topoisomerase I, and 6 plant extracts show potentially effects. There were extracts from root and leaf of *Rhinacanthus nasutus*, whole plant of *Grangea maderaspatana*, caudex of *Stephania suberosa*, rhizome of *Curcuma longa* and *Curcuma zedoaria*. Interesting, the extract of *G. maderaspatana* has never been reported for their topoisomerase I inhibitory activity before. Bioassay-guided fractionation was performed on an ethanolic extract of *G. maderaspatana* using chromatography techniques. The three most active of top I-targeted compounds were isolated.

The present study aimed to screen top I inhibitory activity from Thai medicinal plants by using yeast cell-based assay, followed by bioassay-guided fractionation of the selected plant, *G. maderaspatana*. Isolation and elucidation of the actives compounds were reported. These results could support the use of Thai medicinal plants as traditional medicine for cancer chemotherapy.