

CHAPTER 1

INTRODUCTION

Senna alata (L.) Roxb., (its previous name was *Cassia alata* L.) is a plant belonging to the family Leguminosae. It has a number of common names, including Chum-Het-Thet (Thai), Ringworm senna, Ringworm bush, Candle bush or Candlestick senna (English) (Medicinal Plant Research Institute, 2002; เต็ม, 2544).

In Thailand *S. alata* leaves have long been traditionally used for the treatment of constipation and dermatophyte infections (Farnsworth and Bunyapraphatsara, 1992). Recently, *S. alata* leaves have been approved as a laxative drug in the Thai Herbal Pharmacopoeia 1998 and the Thai National List of Essential Drug 1999 (Subcommittee on the establishment of the Thai Herbal Pharmacopoeia, 1998; National Drug Committee, 2000). Anthraquinone glycosides were demonstrated as the active constituents for the laxative properties (Elujoba *et al.*, 1989), while the aglycones, including aloe-emodin, rhein, emodin and chrysophanol possess antifungal activity (Agarwal *et al.*, 1976; Agarwal *et al.*, 2000). The therapeutic efficacy of *S. alata* leaf extract as a laxative and as a topical antifungal agent in human has been reported (Thamlikitkul *et al.*, 1990; Damodaran and Venkataraman, 1994).

The quality of *S. alata* has been standardized that of hydroxyanthracene derivatives, calculated as rhein-8-glucoside not less than 1.0 %w/w as mentioned in the monograph (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1998). However, poor quality of *S. alata* leaves due to the content of hydroxyanthracene derivatives lower than the standard value is still be a major problem in the production of herbal medicines from *S. alata*. Several factors influence the quality of *S. alata* leaves, including cultivation and harvesting processes. Most raw material of *S. alata* leaves are obtained from naturally growing plants, and the quality of these are dependent on soil and environmental conditions. In addition,

a difference in quality is also due the period of harvesting and the growth stage of the plants (Panichayupakaranant and Intaraksa, 2003).

Plant tissue cultures provide an alternative method of production, instead of using the whole plant or total chemical synthesis. Most plant tissue culture studies aim to establish tissue cultures that are capable of producing high amount of naturally occurring secondary metabolites. Numerous studies with cell cultures from various plants have been published describing the production, in rather high concentration, of many different secondary metabolites. Many cases have been reported where the production in plant tissue cultures was considerably higher than in the intact plants, e.g. hyoscyamine and scopolamine production by *Datura metel* root culture (Cusido *et al.*, 1999) and naphthoquinone production by *Sesamum indicum* hairy root culture (Ogasawara *et al.*, 1993). Moreover, some plant tissues cultures can produce new compounds for example new indole alkaloid formation in *Rauvolfia serpentine* hairy root culture (Sheludko *et al.*, 2002a; Sheludko *et al.*, 2002b).

The use of plant cell cultures as an alternative source of plant constituents has a number of advantages, in particular, independence from geographical, climatic and political environments (Fowler, 1983). In addition, with plant cell cultures, it may be possible to optimize growing conditions, minimise space requirements, achieve a more consistent quality and recover the products more easily (Shargool, 1982). The plant cell cultures also provides an elegant system for elucidating biochemical mechanisms involved in secondary metabolite biosynthesis and degradation, with the inherent questions of storage of intermediates, end products and catabolites. In contrast to intact plant, cell cultures allow an easier analysis of the mechanisms involved in vacuolar influx and efflux as well as translocation of products into the extracellular compartment (Charlwood and Rhodes, 1990). Investment in plant cell culture research should be continued because we cannot rely on current natural environment to remain unchanged in the future.

It has been reported that *S. alata* tissue cultures capable of producing emodin have been established (ฐิติรัตน์ และ นมน, 2543). However, the content of emodin

produced by these tissue cultures was not determined. As part of our interest in the use of tissue cultures as an alternative source of plant chemicals, the tissue cultures of *S. alata* were established. Various techniques, including selection of high yielding plants and medium manipulation, were examined in order to increase anthraquinone production. A HPLC system for quantitative analysis of each anthraquinone was developed. The time courses of growth and anthraquinone production of *S. alata* cell cultures were also studied.

The aims and objective of the present study were therefore on follow:

1. Selection of high anthraquinone yielding plants
2. Establishment of *S. alata* tissue cultures and medium manipulation
3. Study on time course of growth and anthraquinone production
4. Comparison of anthraquinone production between tissue cultures that were established from high yielding and low yielding plants and the intact plants