CHAPTER 1
INTRODUCTION

Plant has been used for many purposes besides providing food along with human history. For example, we use plant extracts for medical treating ailments and cloth dyeing for long time. It has been reported that plant alkaloids such as strychnine, brucine and quinine were first isolated in crystalline forms in the laboratory of Pelletrier and Caventou in France during 1817-1820 (Cordell, 1981). As plants produce a wide array of chemical compounds not found in other organisms, interest was taken in the chemical constituents of plants in the very early days of modern science.

Plant chemicals can be classified as primary or secondary constituents, depending on whether or not they have an essential role in plant metabolism. Primary constituents of plants which are universally present in all plants include common sugars, protein amino acids, purines and pyrimidines of nucleic acids, and pigments such as chlorophylls. Secondary constituents make up all the remaining plant chemicals from alkaloids to terapenoids and acetogenins to phenolics. The vase varieties also represent substances which do not appear to have an essential role in metabolism and which vary in their distribution from plant to plant.

There are so many types of medicinal plant with high potential for production of secondary compounds that can be used to remedy diseases both direct and indirect. For example, herbal plants have been well known from traditional folk knowledge as medicinal plants used for protection and treatment of diseases. Several parts of the plant such as stem, branch, leaf, flower, fruit and root has been reported for medicinal activity (Sarlamp *et al.*, 1996). Many serious diseases which cannot be cured by novel medicine were reported to be successfully recovered by the tradition medicinal plant especially in the rural area.

Various medicinal plants, including herb, are common species in Thailand and can be found almost everywhere. Many herbs have scientifically studied to be placed onto global market base on the curing properties. This objective is strongly supported by the Ministry of Public health (Phurimsak *et al.*, 2005). However, many local medicinal plants are not fully investigated by researchers and their medicinal function has not been reported (Prayoonrat, 1993).

Plookao, *Houttuynia cordata* Thunb., belongs to family Sauruaceae. It is a perennial herb with stoloniferous rhizome. There are two distinct chemotrypes of this plant (Tutupalli *et al.*, 1995) but there is only one chemotypes in Thailand. Plookao is an ethnobotanic species of the northern part of Thailand. The plant has smell like fishwort. It was part of the local disc and has been used especially for human therapheutic in the northern area. Major application includes reduction of inflections disease, cancer, and tumor has also reported for the plant extract. Therefore, Plookao is belonged to a group of high potential plants for drug development in Thailand.

Plant tissue culture or *in vitro* propagation is aseptic culture for propagation of any plant *in vitro.* Plant regenerated form this method will be free from microorganism. Moreover, propagation of plant via tissue culture techniques is a rapid plant propagation method. This is an important and a useful technique for plant multiplication especially for commercial varieties. This method, under the control of both physical and chemical conditions, produces large numbers of high quality plant material within a short period of time (Agriculture forestry and fisheries, 2007).

Major advantage of the micropropagation method is the true to the type of mother plant. The plant seedlings with pest and disease free can be generated using the *in vitro* propagation technique (Rai foundation, 2008). Plant cell and tissue cultures can be established routinely under sterile conditions from explants, such as plant leaves or stems. Plant tissue culture is then central to the innovative areas of applied science, including plant biotechnology and agriculture (Ramachadra and Ravishankar, 2002).

Plant tissue culture is also a method required for secondary metabolite production especially for the human therapheutic chemicals. Because plant secondary metabolites, produced by intact plants, can be used as an important source of active pharmaceutical compounds but it is taking long time of production. In general, after

preliminary study of a plant tissue culture, strategies to improve production of secondary metabolites in cultured plants can be considered. In theory, strain improvement, methods for the selection of high-producing cell lines, and medium optimizations can lead to an enhancement in secondary metabolite production. One of the main problems encountered unsatisfied product is the lack of basic knowledge of the biosynthetic routes, and mechanisms responsible for the production of plant metabolites. In addition, the productivity of the desired metabolites is limited by the lack of particular precursors. For this reason, biotransformation using an exogenous supply of biosynthetic precursors may improve the accumulation of compounds. Feedback inhibition of metabolic enzymes as well as inhibition of membrane transport can be eliminated by the accumulation of synthesized products in a second phase introduced into the aqueous medium. Organ cultures often have sites of synthesis and storage of secondary metabolites in separate compartments. Elicitors, compounds triggering the formation of secondary metabolites, can be abiotic or biotic. Natural elicitors include polysaccharides such as pectin and chitosan are also used in the immobilization and permeabilization of plant cells. Immobilization also provides several advantages, such as continuous process operation (Heike and Dietrich, 1994).

The idea of *in vitro* production of medicinal plants has then been proposed for long time but until now plant biotechnology has led to very few commercial successes for the production of valuable secondary compounds compared to other biotechnological fields. Trials with plant cell cultures most often fail to produce the desired products. There has been very limit reported on *in vitro* propagation of Plookao until now. This study aims to propagate Plookao, *Houttuynia cordata* Thunb. by plant tissue culture technique, and to investigate metabolite production of the *in vitro* plant obtained from the micropropagation method compared to Plookao plant generated by traditional method.

Objectives

The aims and objective of this study are :

1. To study the effect of explants (leaf, stem and rhizome) and sterilization method on *in vitro* propagation of Plookao (*Houttuynia cordata* Thunb.)

2. To study the effect of plant growth regulators on regeneration of Plookao

3. To investigate metabolite production of the *in vitro* plant compared to the normal Plookao plant