CHAPTER 1: INTRODUCTION

1.1. *Curcuma zedoaria*

Curcuma zedoaria is a starchy rhizomatous/tuberous from the Zingiberaceae family (table 1.1), commonly known as ginger family. “Ginger” is a general term for members or species of the ginger families. “Curcuma” is the genus name of the rhizomatous herb, of Zingiberaceae. The name was first given by the Linnaeus species Plantarum from the Arabic term meaning yellow colour, probably referring to the colour of the rhizomes (Govindarajan, 1980).

Taxonomic classification of *Curcuma zedoaria*	
Kingdom	Plantae
Subkingdom	Viridaeplantae
Phylum	Tracheophyta
Division	Magnoliophyta
Class	Liliopsida
Super order	Zingiberanae
Order	Zingiberales
Family	Zingiberales
Genus	Curcuma
Species	*Curcuma zedoaria*
Common Name	Zedoary

Table 1.1 Taxonomic classification of *Curcuma zedoaria*

1.1.1. Description and distribution

Curcuma zedoaria is locally known as “kunyit putih” or “temu putih”. It is able to grow up to one and half meters or even more. The leaves are around eighty centimetres long and they usually have a purple-brown flush along the midrib on both surfaces of the leaf.

The rhizomes are frequently confused with those of *Curcuma aeruginosa* because both are of a similar colour (yellow). However, they can be distinguished easily by conducting a cross section on the rhizomes of the mature plants of *Curcuma aeruginosa* which are slightly dark purplish. In comparison, the colour of the rhizomes of *Curcuma zedoaria* is pale yellow or white. The rhizomes of *Curcuma aeruginosa* are highly aromatic due to the high amount of 1, 8-cineol as 25.20% (Ibrahim *et al.* 2003).

Curcuma zedoaria grows mainly in the East-Asian countries including China (called Er-chu in Chinese), Vietnam, India, Bangladesh, Indonesia, Malaysia (can be found at Kuala Selangor, Teluk Intan; Perak, Labis; Johor, and Pahang) and Japan (Islam *et al. 2005*; Tipthara *et al.* 2007*)*.

Picture 1.1 *Curcuma zedoaria* plant

1.1.2. Chemical and molecular specification

Curcuma spp. contains turmerin (a water-soluble peptide), essential oils (such as turmerones, atlantones and zingiberene) and curcuminoids including Curcumin with the formula: [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-Dione] and the curcuminoids can be defined as phenolic compounds derived from the roots of Curcuma spp. (Sharma *et al.* 2005). Curcumin (diferuloylmethane) which is also called the Indian saffron (Aggarwal *et al*. 2007*)* is a low molecular weight polyphenol, first isolated almost two centuriesago (Aggarwal *et al*. 2007), and first chemically characterised in 1910, that is generally regarded as the most active constituent and comprises 2–8% of most extract preparations (Sharma *et al*. 2005).

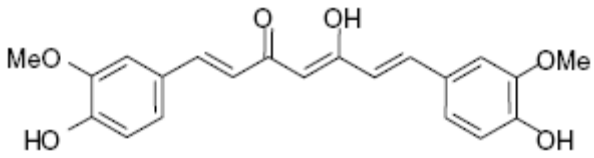

Figure 1.1 Chemical structures of Curcumin

It has been reported (Aggarwal *et al*, 2007) that curcuminoids exist in some Zingiberaceae species such as *Curcuma zedoaria* (White turmeric), *Curcuma longa* (Turmeric), *Curcuma mangga* (Manogo ginger), *Curcuma aromatica* (Wild turmeric), *Curcuma xanthorrhiza* (Ubat maaju), *Costus speciosus* (Crepe ginger), *Zingiber cassumunar* (Cassumunar ginger) *Etlingera elatior* (Torch ginger) and *Curcuma Phaeocaulis* (Aggarwal *et al*. 2007).

1.1.3. *Curcuma zedoaria* and medicine

Curcuma zedoaria has been used traditionally in many countries especially in South-East Asia as a folk medicine for many centuries as a valuable medicinal plant (Wilson *et al. 2005*). In the traditional way, the dried rhizomes of *Curcuma zedoaria* were selected to make drinks or to be extracted as medicine (Maua *et al.* 2003). *Curcuma zedoaria* rhizome extracts which contains Curcumin have been used to treat stomach diseases, blood stagnation, hepato protection, diarrhea, coryza, dermatosis disorders and rheumatism and promoting menstruation as a traditional medicine (Chen *et al.* 2008). Antimicrobial activity (Loc *et al.* 2005), anti-inflammatory antihepatotoxic, neuroprotective activity and cytotoxic effects against human ovarian cancer cells are all regarded as abilities of Curcumin productions from *Curcuma zedoaria* and furthermore, zedoary natural products are used as spices, tonics (Islam *et al.* 2005) and also in perfumery with great luxurious foliage that has high commercial value in floriculture (Islam *et al*. 2005) the rhizomes are also used in food industry as condiment and dye (Loc *et al.* 2005).

There have been many reports on the anti-allergic effects of some plants in the Zingiberaceae family, such as *Curcuma longa* and *Curcuma zedoaria* for treatment of itching and other skin diseases, whereas *Curcuma zedoaria* has been used as a substitute for *Curcuma longa* and has recently been reported to show anti-allergic activity as well (Tewtrakul and Subhadhirasakul, 2007).

Plants containing Curcumin have a range of medicinally active chemicals with more than 500 particular activities in animal systems from more than hundred particular secondary metabolites (Cousins *et al.* 2007).

1.2. Micropropagation

The propagation of this plant species has been only performed through rhizomes (Loc *et al.* 2005). Since Curcuma species are plants from tropical and subtropical regions, it is difficult for the rhizomes to elicit plant formation during the winter season (Miachir *et al.* 2004).

Basically, it is not normal for *Curcuma zedoaria* to be improved by breeding because it seldom produces flowers and the seeds do not form. It is propagated in the vegetative way through underground rhizomes at a very low rate. Thus, a tissue culture technique can play an important role to speed up the studies of this plant and it is also a great industrial way to gain the most important medicinal and pharmaceutical targets.

Micropropagation techniques have been used in a wide range in recent years as an important and advantageous tool for rapid propagation and production of several high qualities and disease free commercially important varieties of plant species (Kapoor *et al.* 2008). Micropropagation is a technical term used for tissue culture which can be described as a group of techniques and methods to produce a large number of desired plants which are genetically identical to a parent plant, as well as to one another cells (Raven *et al*. 1999).

Being one of the most important applications of modern biotechnology, it offers several distinct advantages not possible with conventional propagation techniques micropropagation techniques allow rapid and uniform planting material in relatively short periods of time. Plant tissue culture relies on growing plants based on rich nutrient growth substrates devoid of microbes (Kapoor *et al.* 2008).

1.3. Antimicrobial assay

Plants have been a rich source of medicines because they produce a wide array of bioactive molecules (Ripa *et al*, 2009), and plant extracts are used in the treatment of bacterial, fungal and viral infections (Kuetea *et al. 2009*), this is the reason why people from all over the world follow herbal products for treating different diseases and health problems before resorting to modern medicine (Rout *et al.* 2000).

Herbal medicinal products contain several compounds that can be exploited by human to prevent microbial invasive diseases and illnesses and 40% or more of the pharmaceuticals in the Western countries are already derived or at least partially derived from natural sources; these herbal products have been a major source for drug development as well (Rout *et al.* 2000). Herbal medicine can treat and prevent diseases. These treatments are safe as they are natural (Stickel and Schuppan, 2007).

Plants species of the Zingiberaceae family has been reported for their antimicrobial potentials as well (Aggarwal *et al.* 2007; Niamsa and Sittiwet, 2009; Wilson *et al*. 2005; Chen *et al.* 2008).

After the discovery of antibiotics and different types of antimicrobial peptides or compounds, numerous methods have been developed to test these compounds or chemicals against various organisms (Chen *et al.* 2008).

In the beginning, there were only a few methods for testing the antimicrobial abilities of the herbs; bacteria were incubated with these compounds and the rate of decrease in viable counts was monitored. More efficient methods were sought day after day.

The most successful methods simply exhibited the formation of inhibition zones by an antibiotic in a field of bacteria growing on agar. The inhibition zones were physically measured to determine the antimicrobial activity of the antibiotic (Toit and Rautenbach, *2000*).

Assays for the detection of antimicrobial activity in natural products have become increasingly refined (Smith *et al*, 2008) and there are many biological assay techniques that have been developed to monitor and measure the antimicrobial activity of natural compounds (José *et al. 2006*).

Agar diffusion tests which are actually standard test methods are used to determine the activity of antimicrobial agents or the sensitivity of micro-organisms to such as the agents used in this study. Advantages of agar diffusion tests are the simple and cost-saving actability (Volk, *2008*).

1.4. Aim of the study

This study is made of two major parts; 1.Micropropagation and 2.Antibacterial activity test. In the first part of the project, a tissue culture test (micropropagation) is performed in order to produce several plants and following that, in the second part, a comparison of antibacterial activity of *Curcuma zedoaria* extracts is made between *in vitro* and *in vivo* plant to determine if there is any mentionable deference between the *in vitro* and the *in vivo* system. Effect of hormones on antibacterial activity will be discussed.

The results are valuable for medicinal usage in the industrial field.

Usage of tissue culture in the *in vitro* system (micropropagation) can increase the amount of essential compounds which have antibacterial characteristics in a shorter time compared to the *in vivo* system and consequently save time and are economical in the industry.