# INTRODUCTION

There is a global epidemic of obesity and diabetes. The prevalence of obesity is escalating, and insulin resistance resulting from increased (predominantly visceral) adipose tissue mass has been identified as a key factor that could drive parallel rises in diabetes mellitus (DM) prevalence (Arner, 2003). It is estimated that more than 1.6 billion adults are overweight and 284 million people are diabetic worldwide and 97% of all cases of DM are caused by excessive weight (Stewart et al., 2008). Dietary fat intake has been shown to be important in the development of human obesity, essentially resulting from imbalanced energy intake and expenditure while DM, a metabolic disorder with manifestations of hyperglycemia and hyperinsulinemia, is one of the commonest chronic diseases worldwide. It is also considered as an important risk factor related to the development of different cardiovascular diseases, namely hypertension and stroke (Seto et al., 2009). Recently, there are experimental studies showing that this metabolic disorder can be associated with increased oxidative stress (Ming et al., 2009) with subsequent development of inflammation in our body.

The current treatment for obesity and diabetes involves exercise, diet and a wide range of pharmaceutical drugs. Exercising and dieting, however, are proven to be successful in just a small minority of population. Thus, there is a clear need for effective pharmaceutical intervention. The most effective drug treatment for obesity that is currently available in the market includes fenfluramine, phentermine (Clapham et al., 2001), orlistat (Xenicol), which reduces intestinal fat absorption through inhibition of pancreatic lipase and sibutramine (Reductil), which is anorectic or appetite suppressant (Yun, 2010). These drugs have shown side-effects, including high blood pressure, dry mouth, constipation, headache, valvular heart disease and insomnia (Sloveck et al.,

2008). Thus, doubts have been raised on the long-term sustainability of these weight reduction drugs. Besides that, there are also several drugs that can be found in the market to treat type DM. Besides insulin, other oral hypoglycaemic drugs that are currently used to treat DM include sulphonylurea derivatives, biguanides, thiazolidinediones and alpha glucosidase inhibitors. However, these agents also have undesirable side effects such as thiazolidinediones may induce obesity, osteoporosis and sodium retention. Meanwhile, sulphonylurea derivatives may cause hypoglycaemia. The only available drug that can prevent hyperglycemia without increasing adiposity is biguanide (metformin) but metformin may put patients at risk of developing lactic acidosis (Hamza et al., 2010).

The cost of treating obesity and diabetes and associated complications exceeds $100 billion per year. Due to the multiplication of diabetes and obesity worldwide, a great consideration is given to the disease by health care managements both at national and international levels (Umar et al., 2010). To date, many people are surprised to find natural derived products still playing an important role as sources of medicine in preventing obesity and diabetes.

Natural product preparations have historically been the major source of pharmaceutical agents. Many pharmaceutical agents have been discovered by screening natural products from plants and microorganisms. Analysis of Food and Drug Administration (FDA) new-drug approvals from 1981 to 2002 reveals that natural products continued to play a pivotal role during that time, even if the industry had turned to other discovery strategies. Indeed, more than 90% of current therapeutic classes are derived from a natural product prototype and interestingly, even today, roughly two-thirds to three quarters of the world's population relies upon medicinal plants for its primary pharmaceutical care (McChesney et al., 2007). The belief that

natural medicines are much safer than synthetic drugs has gained its popularity in recent years and led to tremendous growth of phytopharmaceutical usage (Bhattaram et al., 2002). Several screening procedures for biological activities of natural sources including microorganisms and fungus have been carried out in the search for new anti-obesity and anti-diabetes agents (Phillipson, 2001; Newman et al., 2003).

The number of fungal species on earth is estimated to be 1,000,000-1,500,000, but only less than 10% species are known. Mushrooms make up a vast and yet largely untapped source of powerful new pharmaceutical products (Miles & Chang, 1997). Mushrooms have been part of the normal human diet for thousands of years and, in recent times, the amounts consumed have risen greatly, involving a large number of species. Mushrooms are valuable health food since they contain high amount of essential fatty acids, proteins, vitamins, minerals and low in calories and fats (Jayakumar et al., 2011). Edible mushrooms have been used to maintain health, increase longevity and consumed as food (Manzi et al., 1999; Cimerman, 1999). Nowadays, mushrooms are undoubtedly consumed for their nutritional and medicinal properties such as inhibition of platelet aggregation, reduction of blood cholesterol concentrations, prevention or alleviation of heart disease, reduction of blood glucose levels and also prevention or alleviation of infections caused by bacterial, viral, fungal and parasitic pathogens. Attempts have been made in many parts of the world to explore the use of mushrooms and their metabolites for the treatment of variety human ailments (Jose & Janardhanan, 2000). There are various classes of primary and secondary metabolites in mushrooms and they exhibit significant antimicrobial, antiviral and antitumor activities (Liu et al., 2009). Therefore, they represent a valuable source of novel chemotherapeutic agents. To date, there are several natural product agents that have been identified in mushrooms such as grifolin from *Grifola frondosa* (Ye at al., 2005) and suillin from

*Suillus placidus* (Liu et al., 2009) which are used to treat tumor meanwhile lentin from *Lentinus edodes* (Ngai & Ng, 2003) is used as an antifungal agent.

In Malaysia, the widely cultivated 'edible fungal food' is the genus *Pleurotus* which comprises about 40 species and is commonly referred to as "oyster mushrooms". Oyster mushrooms have been discovered to have definite nutritive and medicinal values and are mostly popular in Oriental regions such as China and Japan. *Pleurotus* species are widespread throughout the hardwood forests of the world that host the most diverse varieties in temperate climates (Cimmerman, 1999). Among the different types of mushrooms of the genus Pleurotus, *Pleurotus sajor-caju, Pleurotus ostreatus, Pleurotus citrinopileatus* and *Pleurotus florida* are reported as commonly available edible mushrooms with medicinal properties (Pramanik et al., 2007). According to Stamets (2005), *Pleurotus* is a good source of non-starchy carbohydrate, has high content of dietary fibers and contains moderate quantities of good quality proteins with most of the essential amino acid, minerals and vitamins (Bano & Rajarathnam, 1988). This species have been shown to modulate the immune system, have hypoglycemic activity, anti-trombotic effect, lowers blood pressure and blood lipid concentrations and it is also able to inhibit tumor growth, inflammation and microbial action (Chang, 1993). Since *Pleurotus* cultivation is spreading rapidly in various parts of the world, thus giving it a special status as a useful food for the future.

*Pleurotus sajor-caju* is a delicious edible mushroom, which was first found by an Indian scholar, Yan Dai Ke, at the foot of the Himalayas which was then distributed to many other countries throughout the world (Pramanik et al., 2005). Currently, it is cultivated all over the world. This mushroom contains good quality proteins, vitamins such as $B_1$, $B_2$, and C and very little lipid or starch. This mushroom is able to reduce the cholesterol level in blood. Hence, it is considered as an important health tonic

(Pramanik et al., 2007). Interest in the chemical composition of fruiting bodies has increased throughout the years. There are only few reports on the chemical constituents of *P. sajor-caju*, which were conducted by Caglarirmak (2007) and Chu et al., (2005). Furthermore, these compounds were not correlated to any biological activities that may contribute to the medicinal properties described in the literature. Besides that, Schneider et al., (2011) have reported that *Pleurotus* species might be ideal for dietary prevention of hyperlipidemia due to their low fat and high soluble fiber contents and antioxidative and anti-inflammatory properties. As an example, *n*-3 fatty acids may improve blood serum status and lovastatin could lead to a lipid lowering and anti-diabetic potential (Gunde-Cimerman & Cimerman, 1995). Thus incorporating oyster mushroom in a high-fat diet was first recommended by practitioners of Oriental medicine.

Correlations between these global epidemics (obesity and diabetes) have also encouraged investigation into potential biochemical and molecular links between the related impairments in lipid and glucose homeostasis and the effects *P. sajor-caju* in preventing these metabolic disorders.

**Objectives of the study:**

The objectives of the present study were to:

a. isolate and identify the secondary metabolites and polysaccharides in the fruiting bodies of *P. sajor-caju* by (i) preparation of ethanol aqueous extract and hot aqueous extract*;* (ii) fractionation of ethanol aqueous extracts and hot aqueous extract using column chromatography and precipitation methods and (iii) isolation and identification of compounds from the fractionated extracts using various spectroscopic techniques.

b. screen for antioxidant activities and total phenol content in *P. sajor-caju* extracts using different antioxidant assays namely (i) ferric reducing antioxidant power assay (FRAP); (ii) β-carotene bleaching assay; (iii) trolox equivalents antioxidant capacity assay (TEAC) and (iv) lipid peroxidation assay and correlating the activities with the compounds identified in *P. sajor-caju*.

c. study the effects of *P. sajor-caju* extracts on anti-diabetic potential, lipid lowering activity and oxidative stress via assessment of (i) lipogenesis activity; (ii) lipolysis activity (iii) oxidative indices and (iv) gene expression in 3T3-L1 adipocyte cell line.

d. study the effects of polysaccharide extract of *P. sajor-caju* on (i) metabolic changes; (ii) oxidative stress and antioxidative potential and (iii) gene expression in C57BL/6J mice fed a high –fat diet.