ANTI-OBESITY EFFICACY STUDY OF *PHALERIA MACROCARPA*, *HIBISCUS SABDARIFFA*, AND *MORINGA OLEIFERA*

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by

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

α	Alpha
β	Beta
°C	Degree Celsius
µ	Micro
<	Less than
>	More than

LIST OF ABBREVIATIONS

ACEIs	Angiotensin-converting enzyme inhibitors
API	Active pharmaceutical ingredient
ARBs	Angiotensin-II-receptor blockers
BAT	Brown adipose tissue
BMI	Body mass index
CHEST	Centre for Herbal Standardization
CVD	Cardiovascular disorder
DF	Dilution factor
DSC	Differential scanning calorimetry
FDA	Food and Drug Administration
FFA	Free fatty acid
HDL-C	High density lipoprotein cholesterol
HFD	High fat diet
HPLC	High performance liquid chromatography
IC_{50}	50% inhibitory concentration
IDF	International diabetes federation
IDL-C	Intermediate density lipoprotein cholesterol
K_2HPO_4	Dipotassium hydrogen phosphate
KH_2PO_4	Potassium dihydrogen phosphate
LCR	Low capacity runner
LDL-C	Low density lipoprotein cholesterol
LOD	Limit of detection
LOQ	Limit of quantification
NAFLD	Non-alcoholic fatty liver disorder

NCEP ATP III	National Cholesterol Education Program Adult Treatment Panel III
NE	Norepinephrine
NHMS	National health and morbidity survey
NMD	Normal matched diet
p-NPG	*p*-Nitrophenyl- alpha-D-glucopyranoside
p-NBP	*p*-Nitrophenyl-alpha-butyrate
PPAR-g	Peroxisome- proliferator-activated receptor subtype g
RH	Relative humidity
RO	Reverse osmosis
ROS	Reactive oxygen species
ROTAVAP	Rotary vacuum evaporator
RSD	Relative standard deviation
SEM	Standard error of mean
SD	Standard deviation
SD	Sprague Dawley
SGF	Stimulated gastric fluid
SHR	Spontaneously hypertensive rats
SNS	Sympathetic nervous system
STZ	Streptozotocin
SVF	Stromal vascular fraction
TC	Total cholesterol
TG	Total triglycerol
TM	Traditional Medicine
t_R	Retention time
USP	United States Pharmacopeia
VLDL-C	Very low-density lipoprotein cholesterol

WHR	Waist to hip ratio
WHO	World Health Organization
ZDF	Zucker Diabetic Fatty rats
%LOD	Loss on drying

LIST OF APPENDICES

KAJIAN EFIKASI ANTI-OBESITI *PHALERIA MACROCARPA*, *HIBISCUS SABDARIFFA* DAN *MORINGA OLEIFERA*

ABSTRAK

Obesiti adalah keadaan pengumpulan tisu adipos yang berlaku disebabkan oleh pengambilan kalori tinggi di samping pengunaan tenaga yang rendah untuk jangka masa yang panjang. Glukosidasa alfa dan lipase pankreas adalah enzim makanan yang memecahkan polisakarida dan lemak untuk meningkatkan penyerapan. Merencat aktiviti enzim-enzim ini dapat mengurangkan penyerapan karbohidrat dan lipid. Penyelidikan ini bertujuan untuk mengkaji keberkesanan anti-obesiti dan anti-sindrom metabolik *Phaleria macrocarpa (P. macrocarpa)*, *Hibiscus sabdariffa (H. sabdariffa)* dan *Moringa oleifera (M. oleifera)* menerusi potensi ketiga-tiga tumbuhan itu dalam merencat aktiviti enzim pencernaan. Ekstrak akueus *H. sabdariffa* dan ekstrak etanolik 95% *P. macrocarpa* menunjukkan potensi yang lebih tinggi secara signifikan dalam menghalang aktiviti kedua-dua enzim tersebut. Ekstrak akueus *H. sabdariffa* dan ekstrak etanolik 95% *P. macrocarpa* merencat aktiviti enzim glukosidasa alfa dengan IC_{50} 949.88 ± 10.83 µg/mL dan 822.69 ± 17.40 µg/mL masing-masing. Kedua-dua ekstrak ini juga menghalang aktiviti enzim lipase pankreas dengan IC_{50} 167.51 ± 2.7 µg/mL dan 267.43 ± 10.08 µg/mL masing-masing. Kajian preformulasi menunjukkan bahawa ekstrak etanolik 95% *P. macrocarpa* mempunyai tekstur melekit, keterlarutan dalam air dan kestabilan yang rendah. Oleh itu, ekstrak tersebut telah diformulasikan menjadi granul menggunakan β-siklodekstrin pada nisbah 2: 5 menggunakan teknik granulasi basah secara berperingkat yang menunjukkan profil pelarutan dan kestabilan yang lebih baik berbanding dengan penggabungan dengan eksipien lain. Keberkesanan anti-obesiti dan anti-metabolik sindrom *in vivo* granul ekstrak akueus *H. sabdariffa*

(HSA) dan granul ekstrak etanolik 95% *P. macrocarpa* (PME) dikaji dengan pemberian 250 mg/kg dan 500 mg/kg HSA dan PME kepada tikus Sprague Dawley (SD) yang obes akibat aruhan diet lemak tinggi (HFD) + 20% D-fruktosa selama 60 hari. Dua kumpulan kawalan positif telah dirawat dengan 5 mg / kg acarbose dan 30 mg/kg orlistat masing-masing. Keputusan menunjukkan bahawa peratus peningkatan berat badan bagi kumpulan terawat dengan 500 mg/kg HSA (63.58 ± 9.84 g) dan PME (62.15 ± 18.92 g) adalah lebih rendah daripada kumpulan kawalan negatif (63.58 ± 9.84 g). HSA dan PME juga secara signifikan menghalang peningkatan tahap TG, TC dan LDL-C dengan cara bergantung kepada dos (TG-PME 250 mg/kg: p <0.01, TG-PME 500 mg/kg: p <0.001, TC -PME 250 mg/kg: p <0.001, LDL-HSA 250 mg/kg: p <0.001, LDL-HSA 500 mg/kg: p <0.001, LDL-C -PME 250 mg/kg: p <0.001, LDL-C-PME 500 mg/kg: p <0.001), menunjukkan potensi penurunan lipid. Di samping itu, HSA dan PME mengurangkan ketumpatan lemak dalam tisu lemak perut (250 mg/kg HSA: p <0.001, 500 mg/kg HSA: p <0.05, 500 mg/kg PME: p <0.001). Gandingan kesan anti-obesiti dan kesan penurunan lipid HSA dan PME telah disimpulkan disebabkan oleh aktiviti sinergistik sebatian bioaktif yang ada dalam ekstrak tersebut.

ANTI-OBESITY EFFICACY STUDY OF *PHALERIA MACROCARPA*, *HIBISCUS SABDARIFFA* AND *MORINGA OLEIFERA*

ABSTRACT

Obesity is defined as excess adiposity caused by prolonged high caloric intake and low energy expenditure. Alpha glucosidase and pancreatic lipase are dietary enzymes responsible for the breakdown of large polysaccharides and lipids to enhance absorption. Inhibiting the activity of these enzymes minimizes carbohydrate and lipid absorption. Current research aimed to study the *in vivo* anti-obesity and anti-metabolic syndrome efficacy of *Phaleria macrocarpa (P. macrocarpa), Hibiscus sabdariffa* (*H. sabdariffa*) and *Moringa oleifera (M. oleifera)* through their potential in inhibiting digestive enzyme activities. *H. sabdariffa* aqueous and *P. macrocarpa* 95% ethanolic extract exhibited significantly higher potency in inhibiting the activity of the two enzymes. *H. sabdariffa* aqueous and *P. macrocarpa* 95% ethanolic extract inhibited alpha glucosidase enzyme activity with IC_{50} of 949.88 ± 10.83 µg/mL and 822.69 ± 17.40 µg/mL respectively. The two extracts also inhibited pancreatic lipase enzyme activity with IC_{50} of 167.51 ± 2.7 µg/mL and 267.43 ± 10.08 µg/mL respectively. Preformulation studies revealed that *P. macrocarpa* 95% ethanolic extract exhibited sticky texture, low aqueous solubility and stability. Thus, it was formulated into simple granules using β-cyclodextrin at 2:5 ratio using part by part wet granulation technique which showed improved dissolution profile and stability as compared to incorporation with other excipients. The *in vivo* anti-obesity and anti-metabolic syndrome efficacy of *H. sabdariffa* aqueous extract granules (HSA) and *P. macrocarpa* ethanolic extract granules (PME) were studied by administering 250 mg/kg and 500 mg/kg of HSA and PME to High Fat Diet (HFD) + 20% D-fructose induced obese Sprague Dawley (SD)

rats for 60 days. The two positive control groups were administered with 5 mg/kg acarbose and 30 mg/kg orlistat respectively. Results indicate that the percentage body mass gain of 500 mg/kg HSA (63.58 ± 9.84 g) and PME (62.15 ± 18.92 g) treated groups were significantly lower than the negative control group (90.23 ± 5.34). Accordingly, HSA and PME also significantly inhibited the increase in TG, TC and LDL-C level in a dose dependent manner (TG-PME 250 mg/kg: $p<0.01$, TG-PME 500 mg/kg: $p<0.001$, TC-PME 250 mg/kg: $p<0.001$, TC-PME 500 mg/kg: $p<0.001$, LDL-HSA 250 mg/kg: $p<0.001$, LDL-HSA 500 mg/kg: $p<0.001$, LDL-C-PME 250 mg/kg: $p<0.001$, LDL-C-PME 500 mg/kg: $p<0.001$), indicating their lipid lowering potential. Moreover, HSA and PME significantly reduced accumulation of fat in the visceral fat tissue (250 mg/kg HSA: $p<0.001$, 500 mg/kg HSA: $p<0.05$, 500 mg/kg PME< 0.001). The dual anti-obesity and lipid lowering effects of HSA and PME are deduced to be due to synergistic activity of bioactive compounds present in their extracts.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Obesity is a medical condition in which the body is accumulated with excessive fat beyond normal range (Bais *et al.* 2014). It is caused by long term disproportion between total energy intake and expenditure (Lee *et al.* 2010). Metabolic syndrome is defined as a constellation of diseases including obesity, insulin resistance, diabetes mellitus type II, hyperlipidaemia, non-alcoholic fatty liver disorder (NAFLD) and hypertension which elevate the risk of developing cardiovascular disorder (CVD). It is a condition which was first coined in the West and reports of its diagnosis has spread to the Asian countries along with western lifestyle such as consumption of high caloric fast food and reduced physical activity. Obesity is the central factor for the development of metabolic syndrome (Aggoun, 2007; Hermann *et al.* 2004). According to the National Health and Morbidity Survey (NHMS) by the Malaysian Ministry of Health in 2015, the national adult prevalence of overweight and obesity were 30% and 17.7% respectively (Nor *et al.* 2018; NHMS, 2015). Although treatments including dieting, exercising, surgeries, and drugs are available, so far none are thoroughly effective. Most effective anti-obesity drugs are terminated at the preclinical stage itself due to toxicity and threatening side effects (Elangbam, 2009). Currently, pharmaceutical researchers are exploring the potency of natural products for the treatment of obesity.

1.2 Obesity

Generally, a person with BMI above 30 kg/m^2 is said to have developed (Ullah *et al.* 2018). Obesity has to be taken seriously because besides being the most prevalent manifestation for metabolic syndrome, it is associated with many crucial diseases such as cancer and stroke (Padmaja & Naidu, 2014).

1.2.1 Epidemic of obesity

The prevalence of obesity has been increasing worldwide at an alarming rate. According to the World Health Organization, (WHO, 2020) the global obesity prevalence has nearly tripled since 1975. The organization also mentioned that in 2016, 39% (1.9 billion) of adults were overweight and 13% (650 million) were obese worldwide. According to Yoon *et al.* (2006) Asians have higher fat content and are at higher risk of diabetes, high blood pressure and cardiovascular disorder as compared to others with the same BMI. In the year 2014, Malaysia was ranked as the country with highest overweight population among the Southeast Asian countries (Zarkasi *et al.* 2018; Ghee, 2016; Kutty *et al.* 2015) with 42.5% of overweight prevalence (WHO, 2019). Musa *et al.* (2012) reported that Malaysia has the highest prevalence of abdominal obesity compared to other countries with similar ethnic populations, such as China, Hong Kong, and India. In Malaysia, the prevalence of obesity was highest amongst Malays (13.6%) and Indians (13.5%) followed by Chinese (8.5%). Whereas, the indigenous group of "Sabah Bumiputra" had the lowest prevalence of 7.3% (Rampal *et al*. 2007).

1.2.2 Causes of Obesity

1.2.2(a) Environmental and lifestyle factor

Once the number of calories consumed exceeds the number of calories burned, excess fat is stored in the adipocytes. Hypothetically, obesity could be caused by increased food intake, reduced energy expenditure or from a combination of both (Lowell & Bachman, 2003). Mutiso *et al*. (2014) also supported that obesity is associated with poor eating habits and lack of physical activities. Due to recent rapid global development and busy working schedule, people have limited time and choice of healthy food. Hence, easily available fast food is preferred for consumption. However, the bad news is that fast food normally has high caloric content and should ideally be balanced with high physical activity. In contrast, current lifestyle with advanced transportation and occupations with minimum physical movement minimizes energy expenditure and subsequently accelerates the development of obesity.

1.2.2(b) Genetic predisposition

According to Mohamed *et al.* (2014) some individuals are predisposed to obesity. They are 1.67-fold at higher risk of developing obesity compared to normal individuals. Hence, those predisposed to obesity attain the disease more easily compared to others when they are exposed to environmental factors such as high caloric food intake and lack of physical activities. According to Musa *et al.* (2012), Asians, especially South Asians, have an ethnic predisposition to abdominal obesity, hypertension, dyslipidaemia, hyperinsulinemia and glucose intolerance thus, have a greater risk for developing metabolic syndrome. Evidently, Dorajoo *et al.* (2012) confirmed the presence of obesity associated genes (*FTO, FAIM2, PTBP2* and *CADM2*) among the

three major Asian ethnic groups (Malays, Chinese, and Indians). Significant increase in fat mass among children and birth weight among new-borns in relation to height were reported to be due to *FTO* gene (Fawcett & Barroso, 2010). Frayling *et al.* (2007) stated that 16% of adults who are homozygous for *FTO* risk allele weighed 3.0 kg more than those not inheriting the risk allele and had higher risk of developing obesity.

1.2.3 White adipose tissue (WAT) and brown adipose tissue (BAT)

Adipose tissue is a complex endocrine organ which plays a vital role in energy homeostasis. Major fat depots in humans are visceral (intra-abdominal) and subcutaneous fat depots. These depots are composed of two main types of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT stores excess energy in the form of triglycerides which accounts for 90% of the cell volume (Saely *et al.* 2011). It is made up of a single lipid droplet and a variable amount of mitochondria. It is primarily accumulated in the visceral fat tissues (Rosell *et al.* 2014) which is the dominant factor for occurrence of metabolic syndrome (Ritchie & Connell, 2007). The number of WAT increases with obesity development (Garrastachu, 2009). BAT on the other hand, functions to dissipate energy in the form of heat by burning fatty acid to maintain body temperature (non-shivering thermogenesis). Thus, it is significantly high in new born infants and small mammals (Garrastachu, 2009). BAT is made up of several small sized lipid droplets, vascularization and a high number of iron-rich mitochondria which accounts for its brownish colour (Saely *et al.* 2011). Although BAT had long been considered as vestigial in adult humans, recent researches show evidence of BAT presence in adult humans and its role in energy homeostasis (Saely *et al.* 2011; Song *et al.* 2017; Wehrli *et al.* 2007). Increase in BAT is associated with decreased risk of obesity and its related

disorders, in contrast to WAT (Garrastachu, 2009; Rosell *et al.* 2014). In adults, BAT has been found to be distributed throughout the supraclavicular, cervical, axillary, mediastinal, paravertebral, and upper abdominal regions (Wehrli *et al.* 2007). Thus, BAT is receiving great attention among researchers as a potential target to treat obesity and its associated metabolic abnormalities.

1.2.4 Obesity as the most significant risk factor for metabolic syndrome

Abdominal obesity is the most prevalent manifestation for metabolic syndrome (Zainuddin *et al.* 2011; Ram Weiss, M.D *et al*. 2010; Rosario & Isabel, 2010; Després & Lemieux, 2006). Accumulation of visceral fat causes hypertriglyceridemia, insulin resistance, NAFLD, hypertension and consequently develop metabolic syndrome. In other words, an obese person is at higher risk of developing metabolic syndrome and its associated diseases including cardiovascular disorder (CVD).

Adipose tissue is made up of adipocytes and stromal vascular fraction (SVF) consisting of preadipocytes, fibroblast, endothelial cells and immune cells including macrophages, monocytes, and lymphocytes. Initially, adipose tissue was only known as an energy storage organ which stores excess lipid in the form of triglycerides and releases fatty acid during energy deprivation. However, various findings have demonstrated the function of adipocytes as a complex, dynamic and metabolically active endocrine organ which is also involved in glucose and lipid metabolism, appetite control and inflammation regulation (Rens L.J *et al.* 2019). The organ releases hormones, cytokines and chemokines which are collectively known as adipokines. Many of them including tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-4, IL-

6, IL-18, leptin, and adiponectin are involved in energy homeostasis and inflammation (Unamuno *et al.* 2018; Gomez-hernandez *et al.* 2016).

In normal condition, adipocytes contain alternatively activated (M2) macrophages which are involved in tissue repair and secretion of anti-inflammatory cytokines including IL-4, IL-10 and IL-13 (Unamuno *et al*. 2018; Odegaard & Chawla, 2011). However, excess lipid accumulation causes increase in cell size (hypertrophy) and cell number (hyperplasia). In adults, increased in fat mass is primarily attributed to hypertrophy (Jung & Choi, 2014). The hypertrophic stress condition causes insufficient oxygen supply to adipose tissue (hypoxia) and infiltration of M1 macrophages into adipocytes (Unamuno *et al.* 2018; Jung & Choi, 2014). Infiltration of M1 macrophages indicate adipose tissue dysfunction and is a definitive sign of obesity (Jung & Choi, 2014). In humans, significant increase in M1 macrophage infiltration as a consequence of hypertrophy occurs mainly in visceral adipose tissue (Unamuno *et al.* 2018) signifying the role of visceral obesity as the central factor for metabolic disorders. The macrophages secrete pro-inflammatory cytokines including (TNF)-α, IL-1, IL-6, IL-18, and MCP-1 that are associated with increased release of free fatty acid (FFA) (Jung & Choi, 2014). The FFA gets into metabolic tissues including liver and alter the lipid and glucose metabolism (Jung & Choi, 2014; Lumeng & Saltiel, 2011). Several studies have also demonstrated that increase in serum FFA causes insulin resistance and hepatic gluconeogenesis (Y. H. Lee *et al.* 2015; Lumeng & Saltiel, 2011; Wang *et al.* 2011). Taken together, lipid accumulation in adipose tissue causes adipose tissue dysfunction, dysregulated secretion of adipokines and imbalance in glucose and lipid metabolism; linking obesity to other metabolic disorders including hyperglycaemia and NAFLD.

1.2.4(a) TNF-α

TNF-α is a proinflammatory cytokine that leads to the pathogenesis of obesity and insulin resistance. It has been reported that the TNF-α level is positively corelated with the size of adipocytes (Gomez-hernandez *et al.* 2016). Hotamisligil *et al.* (1993) proved that treatment with TNF-α significantly increased glucose uptake in obese rats signifying the role of TNF-α in insulin resistance. Accordingly, deletion of TNF-α increased insulin sensitivity in obese rats (Uysal *et al.*1997) indicating its role in obesity induced insulin resistance. Besides, overexpression of TNF-α induces secretion of other pro-inflammatory cytokines but inhibit the secretion of anti-inflammatory cytokines including adiponectin (Emanuela *et al.* 2012) further elevating obesity association with metabolic syndrome.

1.2.4(b) IL-6 and IL-18

IL-6 and IL-18 are pro-inflammatory cytokines secreted by adipose tissue which are reported to elevate insulin resistance in obese subjects (Fernandez *et al.* 2003; Bastard, 2002). Treatment with IL-6 has shown to induce insulin resistance and hyperglycaemia in humans (Tsigos *et al.* 1997). Similarly, IL-18 level was observed to be significantly higher in obese subjects and reduced significantly upon weight loss (Esposito *et al.* 2002).

1.2.4(c) Leptin

Leptin is a hormone secreted by adipose tissue which is involved in energy homeostasis. It regulates energy balance by inhibiting appetite, reducing food intake and increasing energy expenditure. It also regulates glucose metabolism by improving insulin sensitivity in the muscle and liver besides enhancing β-cell function. However,

excessive secretion of leptin in hypertrophic cells cause leptin resistance and subsequently insulin resistance. Since it promotes pro-inflammatory cytokines secretion and inhibits anti-inflammatory cytokine secretion, increase in leptin level in obese patients further elevates pro-inflammatory cytokines level and deprive anti-inflammatory cytokine level (Gomez-hernandez *et al.* 2016).

1.2.4(d) Adiponectin

Adiponectin is the most abundant adipokine secreted mainly by adipocytes (Doyle *et al.* 2012) and plays important role in increasing insulin sensitivity, regulating food intake, preventing chronic inflammation (Emanuela *et al.* 2012), increasing fatty acid oxidation, and decreasing gluconeogenesis (Divella *et al.* 2019). However, the adiponectin level drops with obesity development (Ghee, 2016). Cross-sectional studies showed that drop in adiponectin level increases the risk of CVD (Gomez-hernandez *et al.* 2016; Xu *et al.* 2010). TNF- α and IL-6 inhibit the adiponectin secretion (Kershaw & Flier, 2017).

1.2.5 Measurement of obesity

Body weight only does not indicate obesity because it does not differentiate between fat and muscle mass. Two means recommended by WHO to evaluate obesity are Body Mass Index (BMI) and Waist to Hip Ratio (WHR) as shown in *Equation 1.1* and *1.2*. Table 1.1 shows the BMI value which was set by WHO in 2000 (D. J. Kumari, 2010). BMI is a measurement which correlates weight and height. WHR is a good measurement to indicate abdominal obesity and increased risk of hypertension, diabetes mellitus type II, and cardiovascular disorder. The classifications of WHR set by WHO (2000) is reported in Table 1.2.

$$BMI = \frac{Mass\ (kg)}{[Height\ (m)]^2} \qquad \textit{Equation 1.1}$$

$$WHR = \frac{Waist\ circumference}{Hip\ circumference} \qquad \textit{Equation 1.2}$$

Table 1.1 BMI values set by WHO (2000)

Classification	**BMI**
Underweight	<18.5
Normal weight	18.5-24.9
Overweight	25-29.9
Class I obesity	30-34.9
Class II obesity	35-39.9
Class III obesity	≥40
Morbid obesity	>40 to 44.9
Super obesity	>44.9

Table 1.2 *WHR* classification set by WHO (2000)

Group	**Waist-hip ratio (*WHR*)**	
	Male	**Female**
Normal	< 0.9	< 0.85
At risk	> 0.9	> 0.85

1.2.6 Currently available treatments for obesity

Although research has been carried out in the past to find an effective approach to treat obesity, so far currently available pharmacotherapy for obesity has limitations including limited number of approved drugs, significant side effects, and limited drug efficacy (Kakkar & Dahiya, 2015). Even the natural way of losing weight such as dieting and exercising do not result in satisfactory weight loss due to human reluctance (Rodgers *et al.* 2012; Dansinger *et al.* 2005). Surgeries available such as bariatric surgery and liposuction are also complicated and leads to various side effects (Rodgers *et al.* 2012) including the need for re-operation due to surgery complications such as abdominal hernias. One third of post-surgery patients also developed gall stone,

anaemia, and osteoporosis as a result of nutritional deficiency (Stern & Kazaks, 2009) with death rate of 1 out of 200 surgeries (Stern & Kazaks, 2009; Morino *et al.* 2007). While, abundant of drugs have been claimed to be efficient in treating obesity, to date, only five anti-obesity drugs are approved by the Food and Drug Administration (FDA) for long term obesity treatment because other effective anti-obesity drugs led to hazardous side effects while orlistat (Xenical), Lorcaserin (Belviq), Phentermine (Qsymia), Naltrexone (Contrave) and Liraglutide (Saxenda) are associated with mild side effects (Daneschvar *et al.* 2016). Table 1.3 shows the list of anti-obesity drugs with significant weight loss that has been approved by FDA and their associated side effects as reported by Daneschvar *et al.* (2016) and Kakkar & Dahiya (2015). Sibutramine (Meridia) was approved by FDA on 1997 and was withdrawn from the market during mandatory post marketing studies despite its efficacy in the treatment of obesity due to increased risk of heart attack and stroke on 2010 (Daneschvar *et al.* 2016). Similarly, Dinitrophenol, Amphetamines, Amonirex, Mazindol and Rimonabant were also withdrawn due to their adverse side effects (Kakkar & Dahiya, 2015).

Table 1.3 FDA approved anti-obesity drugs and their side effects

Anti-obesity drug	Mechanism of action	Effect on lipid profile	Side effects	FDA approval
Orlistat Dosage: 120 mg	Lipase enzyme inhibitor	Reduces TG, TC, and LDL-C level	Flatulence and steatorrhea, kidney stone, pancreatitis, hepatotoxicity, nephrotoxicity	1999
Lorcaserin Dosage: 120 mg	Appetite suppressant	Reduces TC and TG level	Breast adenocarcinoma, depression, astrocytoma, cancer schwannoma	2012

Anti-obesity drug	Mechanism of action	Effect on lipid profile	Side effects	FDA approval
Phentermine/ Topiramate Dosage: 3.75/23 mg and 7.5/46 mg	Appetite suppressant and thermogenesis enhancer	Reduces TC, LDL-C, TG level and increases HDL-C level	Dry mouth, constipation, insomnia, dizziness	2012
Naltrexone/ bupropion Dosage: 32/360 mg	Appetite suppressant	Reduces TC, LDL-C, TG level and increases HDL-C level	Dry mouth, constipation, insomnia, dizziness, diarrhoea, headache, nausea, vomiting,	2014
Liraglutide Dosage: 3 mg		Reduces TC, LDL-C, TG level and increases HDL-C level	Hypoglycaemia, constipation, vomiting, diarrhoea, headache, fatigue, dizziness, abdominal pain	2014

1.2.6(a) Orlistat

Orlistat is a hydrogenated drug form of lipstatin which is produced by *Streptomyces toxitricini*. It was approved by FDA for prescription sale in 1997 (Kakkar & Dahiya, 2015) and was the only FDA approved therapy for long term obesity treatment until Lorcaserin and Phentermine/Topiramate was approved in 2012 (Daneschvar *et al.* 2016). In Malaysia, orlistat is classified as Poison C as stated in the First Schedule of the Poisons List, which can be found in the web portal of the Pharmaceutical Services Programme, Ministry of Health, Malaysia. The drug helps in weight loss by inhibiting the activity of lipase enzymes. Lipase enzyme breaks down large triglycerides into simpler glycerol and fatty acids to ease absorption of lipids. Inhibition of the enzyme activity prevents the breakdown of triglycerides thus minimizing absorption of fat. Consequently, excess fat is eliminated from the body through faeces. By inhibiting the lipase enzyme activity, orlistat is proven to prevent 30% of dietary fat absorption from intestine into the bloodstream (Ado *et al.* 2013).

1.2.6(b) Acarbose

Acarbose is a drug commercially used to treat post-prandial hyperglycaemia. It is a potent inhibitor of alpha glucosidase enzyme activity. The enzyme breaks down large polysaccharides into simpler disaccharides or monosaccharides to ease absorption. When acarbose inhibits the activity of this enzyme, the breakdown and absorption of sugar into the bloodstream can be minimized. Thus, excess sugar can be eliminated from the body through faeces. In Malaysia, acarbose is classified as Poison C in the Poisons List. Although acarbose is a commercialized drug which is highly potent, it is also coupled with several side effects such as abdominal discomfort and diarrhoea (Bachhawat *et al.* 2011). Acarbose is also used as an 'over the counter drug' for the treatment of obesity (Clissold & Edwards, 1988). Özgen *et al.* (2014) investigated the anti-obesity potential of 300 mg/kg acarbose in obese women fed with low-caloric diet. They revealed that women who received 300 mg/kg acarbose treatment in addition to low-caloric diet for 10 weeks lost significantly more weight than women who were fed with low-caloric diet only.

1.3 Current pharmacological research approaches for the treatment of obesity

Obesity is caused by imbalance between total energy intake and expenditure. Excess fat is stored in the body when the calories taken is more than the calories expended. Thus, the current pharmacological approaches for the treatment of obesity aims to decrease energy intake (appetite suppressing), increase energy expenditure (thermogenesis), or inhibit absorption of lipids and carbohydrates.

1.3.1 Appetite suppressing

As the factor causing obesity is increased food intake and decreased energy expenditure, reducing food intake could be an approach in combating obesity and its related disorders. Imbalance between hunger and satiety signals lead to increase in food intake and subsequently increase in body weight. Leptin, insulin and cholecystokinin are hormones which promote (satiety) fullness. In contrast, ghrelin hormone promotes hunger. The level of ghrelin hormone is reported to be higher in fasting individuals. In some cases, the level of leptin hormone in obese subjects are found to be significantly low causing patients to not attain satiety. Thus, an approach to increase satiety causing hormone may be another approach to treat obesity (Sun *et al.* 2016; J. H. Yu & Kim, 2012). However, appetite suppressing may lead to nutrient deficiency.

1.3.2 Thermogenesis enhancement (β3 -adrenoreceptor agonists)

Energy expenditure occurs in the form of physical activities, basal metabolism and adaptive thermogenesis. Physical activities refer to voluntary movement. Basal metabolism is biochemical reactions that take place in the cells to sustain life. Adaptive thermogenesis on the other hand, is alteration of energy expenditure in response to heat due to environmental changes such as exposure to cold or diet alteration (diet induced thermogenesis) (Spiegelman *et al.* 2001). Diet induced thermogenesis is also known as Brown Adipose Tissue (BAT) thermogenesis because it occurs in BAT. The function of diet induced thermogenesis is to prevent the body against obesity and insulin resistance. Diet induced thermogenesis occurs in response to variation in diet intake of a person (energy homeostasis). Everyone has a fat mass ‘set point’. Diet

induced thermogenesis takes place in the Brown Adipose Tissue when the fat composition in the body deviates from the normal range. For instance, a person who gained 10% body weight experienced a significantly higher energy expenditure compared to a person who had never experienced such a body weight gain (Lowell & Bachman, 2003). Sympathetic nervous system (SNS) activates the BAT thermogenesis with the release of norepinephrine (NE) and stimulation of α and β adrenergic receptors (Ueta *et al.* 2012). For the treatment of obesity, drugs called the β -adrenoreceptor agonists are used to initiate the β -adrenoreceptor and increase thermogenesis.

1.3.3 Adipogenesis prevention

Unlike other cells, adipocytes can increase its size by 4 times for fat storage before dividing and differentiating into new preadipocytes. Adipogenesis refers to the cell differentiating process by which preadipocytes differentiate into mature adipocytes. Adipogenesis also causes further hypertrophy and hyperplasia which lead to excess lipid accumulation and consequently obesity (Sun *et al.* 2016). Hence, a pharmacological approach of inhibiting adipogenesis prevents accumulation of excess fat and its associated metabolic abnormalities.

1.3.4 Lipase enzyme inhibition

One of the promising approaches for obesity treatment is by inhibiting fat absorption at the gastrointestinal tract directly. Lipase enzymes are found in abundance at the intestine. It breaks down large triglycerides into simpler monoglycerides and fatty acids to enhance absorption. It is responsible for 50% to 70% of total dietary fat hydrolysis (S. Liu *et al.* 2013). Inhibiting the activity of this enzyme reduces

absorption of fat into the body. Excess fat is eliminated from the body through faeces. Hence, inhibiting the pancreatic lipase enzyme is a good approach as excess fat is eliminated before entering the circulatory system itself.

1.4 Metabolic syndrome

Metabolic syndrome is defined as a cluster of diseases which is associated with the risk of developing cardiovascular disorder (CVD) (Cleeman, 2018; Zainuddin *et al.* 2011). CVD is reported to account for 27% of deaths in South East Asia (Misra *et al.* 2019). Generally, a person who has obesity and any two or more of the following abnormalities is said to have metabolic syndrome; hypertension, hyperglycaemia, low HDL cholesterol, hyperlipidaemia (Eckel *et al.* 2005). According to Aggoun (2007) and Grundy *et al.* (2004), compared to a normal person, a person who has metabolic syndrome is at a two-fold higher risk of developing CVD.

1.4.1 Prevalence of metabolic syndrome

Zainuddin *et al.* (2011) reported that Asians are highly predisposed to metabolic syndrome compared to other ethnicities. The latest prevalence of metabolic syndrome in Malaysia is 42.5% (Ching *et al.* 2018). The occurrence of metabolic syndrome increases as the severity of obesity increases (Ram *et al.* 2010). According to Musa *et al.* (2012) and W. S. Tan *et al.* (2011) the pervasiveness of metabolic syndrome rises with age. The development of metabolic syndrome is 15% higher in patients above the age of 40 compared to the younger ones (Ghee & Kooi, 2016). For every year increase in age the risk of metabolic syndrome increases by 3% (Chee *et al.* 2014). Musa *et al.* (2012) reported that the epidemic of metabolic syndrome was highest among Indians

(51.9%), followed by Malays (43.9%) and Chinese (42.1%). They also reported that the occurrence of metabolic syndrome is higher in females compared to males.

1.4.2 Clinical diagnosis of metabolic syndrome

In 2001, the National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) formulated the definition of metabolic syndrome according to Table 1.4 (Han & Lean, 2017; Eckel *et al.* 2005). Han & Lean, (2017) reported that if BMI is ≥30 kg/m^2 then waist circumference can be assumed to be above treatment level.

Table 1.4 Criteria for diagnosis of metabolic syndrome as defined by NCEP ATP III

NCEP ATP III, 2001
Any three of the following abnormalities: ▪ Increased visceral fat: Waist circumference >102 cm for men Waist circumference > 88 cm for women ▪ Triglycerides level ≥ 1.7 mmol/L (150 mg/dL) ▪ HDL-C level < 1.03 mmol/L (40 mg/dL) for men ▪ HDL-C level < 1.29 mmol/L (50 mg/dL) for women ▪ Blood pressure ≥ 130/85 mmHg ▪ Plasma glucose level ≥ 6.1 mmol/L (110 mg/dL)

1.4.3 Diseases associated with metabolic syndrome

1.4.3(a) Hyperlipidemia

Hyperlipidaemia is a medical condition that occurs when any of the blood lipids (or lipoprotein) level elevate beyond normal range (Rasool Hassan, 2013). It is a common medical condition caused by alteration in the lipid and (or) lipoprotein metabolism (Rasool Hassan, 2013). Hyperlipidaemia can be generally classified into primary hyperlipidaemia that is caused by genetic factors; and secondary hyperlipidaemia that is originated as a result of other diseases including diabetes, chronic alcoholism and

metabolic syndrome (Shattat, 2014). Lipid refers to the entire class of fat including triglycerides, fatty acids, cholesterol and phospholipids. Whereas, lipoproteins are macromolecules that allow lipids to dissolve in aqueous plasma for transportation, storage and absorption (Nelson, 2013). It is composed of hydrophobic lipids and hydrophilic protein. The five major classes of lipoprotein which are classified according to their density are high-density lipoprotein-cholesterol (HDL-C), intermediate-density lipoprotein-cholesterol (IDL-C), low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C) and chylomicrons (Nelson, 2013).

1.4.3(b) Hyperglycemia and insulin resistance

Diabetes mellitus is a chronic hyperglycaemic condition that occurs over a long period of time due to several metabolic disorders. It is an abnormal condition that takes place when the β cells do not produce enough insulin (diabetes mellitus type I) or when the cells fail to respond normally to insulin (insulin resistance) leading to decrease in insulin production (WHO, 2018). Glucose homeostasis is a body's natural mechanism to maintain the plasma glucose level. Insulin and glucagon are hormones secreted by the pancreatic α and β cells respectively to balance the plasma glucose level. When the plasma glucose level is high, insulin decreases the glucose production in the liver and increases glucose uptake by the skeletal muscle and peripheral tissue besides enhancing glucose storage in the form of glycogen in the liver and muscle. Likewise, when the plasma glucose level is low, glucagon increases liver glucose production, and permits glycogen breakdown into glucose. Holding an important role in the glucose homeostasis, insulin resistance and insulin deficiency leads to a state of hyperglycaemia. Diabetes mellitus is also reported to be associated with other

metabolic abnormalities including hyperlipidaemia, hypertension and obesity (Gonzalez *et al.* 2006).

1.4.3(c) Hypertension

Hypertension is defined as having systolic blood pressure of 140 mmHg and/or diastolic blood pressure of 90 mmHg (Kuhlemeier, 1994). Optimal blood pressure is systolic blood pressure 120 mmHg or less and diastolic blood pressure 80 mmHg or less. The hypotensive potential of several plants has been previously investigated and validated including aqueous stem bark extract of *Musanga cecropioides* (Adeneye *et al.*, 2006), aqueous seed extract of *Persea americana* (Anaka *et al.* 2009), *Salvia miltiorrhiza* and *Pueraria lobate* (Ng *et al.* 2011) in Sprague Dawley (SD) rats.

1.4.3(d) Non-alcoholic fatty liver disorder (NAFLD)

Obesity is associated with dysregulation of lipid metabolism (Ok *et al.* 2013) which dominantly takes place in the liver (Nguyen *et al.* 2008). The liver is made up of hepatocytes which regulates blood lipid level by synthesizing lipoprotein, phospholipids and cholesterol. It also converts excess carbohydrates to fatty acid and triglycerides which are then stored in the adipose tissue (lipogenesis). Dysregulation in the lipid metabolism causes accumulation of excess fat in the form of lipid droplets in the hepatocytes which results in hepatic steatosis. Micro-vesicular steatosis is accumulation of excess fat in the hepatic vesicles which displaces cytoplasm. Macro-vesicular fatty change on the other hand, is a severe form of steatosis in which fat accumulates in the vesicles to the extent which nucleus is distorted. Hepatic steatosis is an abnormal retention of fat in the liver which is indeed a hallmark of NAFLD and consequently metabolic syndrome and other metabolic related disorders. Altunkaynak

& Özbek, (2009) reported that metabolic disorders such as type II diabetes mellitus, insulin resistance, and hyperlipidaemia were significant in most patients with liver associated problems. 70 to 90% of NAFLD were diagnosed in obese and diabetic patients (Ramgopal *et al.* 2014). Previous studies have also reported that high fat diet (HFD) induces development of NAFLD (Altunkaynak & Özbek, 2009; Kameshwara, *et al.* 2013; Ramgopal *et al.* 2014) and can be estimated through liver weight measurement and histopathology studies (Meriga *et al.* 2017; Nursyuhana *et al.* 2016; Nurul Shazini Ramli *et al.* 2016).

1.4.4 Currently available treatment for metabolic syndrome

The fundamental treatment for metabolic syndrome is lifestyle changes including diet alteration, minimizing physical inactivity, daily moderate exercising and avoiding smoking. Increased serum FFA level has been proven to play important role in the development of insulin resistance (Riccardi *et al.* 2004). Thus, diet alteration by reducing saturated fat intake maybe useful in improving insulin sensitivity (Bianchi *et al.* 2007). Besides that, exercising improves insulin sensitivity by which the effect persisting for 24 to 48 hours and disappears within 3 to 5 days upon exercising. Thirty minutes of daily moderate physical activity has thus been recommended to improve insulin sensitivity (Bianchi *et al.* 2007).

As metabolic syndrome is a collection of several diseases and thus is associated with different mechanisms, it is hard to develop a specific drug to treat metabolic syndrome as a whole. In fact, clinical trials of drug treatments for metabolic syndrome are difficult to design due to complexity of the syndrome (Zwieten *et al.* 2006). However, several drugs appear to improve more than one component of metabolic

syndrome. Hence, they are prescribed for patients having a combination of diseases. For instance, metformin improves glucose tolerance and lipid profile. Pioglitazone and rosiglitazone are oral antidiabetic drugs which induces insulin sensitivity by activating the peroxisome- proliferator-activated receptor subtype gamma (PPAR-gamma) and can also be prescribed for insulin resistant metabolic syndrome patients (Zwieten et al. 2006). Accumulation of visceral adipose tissue is a major factor responsible for various diseases associated with metabolic syndrome, including insulin resistance. Thus, anti-obesity drugs including orlistat are used in the treatment of obesity and metabolic syndrome because weight loss is the preliminary treatment for metabolic syndrome. In fact, 5 to 10% of weight loss contributes to major positive changes in all metabolic risk factors by 50% (Han & Lean, 2017).

Table 1.5 summarises some currently prescribed drugs for metabolic syndrome associated diseases. Since metabolic syndrome is a multigenic condition caused by alteration in various metabolic factors, utilising plant extracts consisting of abundance of bioactive compounds could be a more reliable approach than relying upon single drugs available in the market which often targets a single site of action.

Table 1.5 Currently prescribed drugs for the treatment of metabolic syndrome associated diseases according to Bianchi *et al.* (2007)

	Metabolic syndrome associated risk factors			
	Obesity	**Hyperglycaemia**	**Hyperlipidaemia**	**Hypertension**
Drugs	Orlistat, sibutramine	Metformin, pioglitazone, rosiglitazone	Fibrates, niacin, Statins	angiotensin-converting enzyme inhibitors (ACEIs), angiotensin-II-receptor blockers (ARBs)
Goal	7 to 10 % of body weight reduction	Heamoglobin A1C (HbA1c) <6.5%	LDL < 160 mg/dl	Blood pressure <140/90 mmHg

1.5 Medicinal plants

Fossil records verified that humans have been using medicinal plants for treatment since at least 60 000 years ago which is around the middle of the Palaeolithic age. The discovery of medicinal plants, their therapeutic values and even toxicity among early humans is reported to be by trial and error method. While seeking for food, early humans consumed a variety of plants including poisonous plants and discovered the physiological effect of each plant on the body (Yuan *et al*. 2016). Upon the advent of writing, the therapeutic values of thousands of plants were recorded and is being used traditionally till date (Thomford *et al*. 2018). Some of the oldest well known traditional medicinal systems in the world which utilize medicinal plants to cure sickness are Ayurveda and Traditional Chinese Medicine. Ample of research including in ethnopharmacological, ethnobotanical, and natural product drug discovery fields uses traditional medicine as a guideline for discovery of therapeutics. Currently, the new trend in drug discovery focuses on the therapeutic activity of plants because of the presence of various bioactive compounds that have been proven to synergistically enhance therapeutic effect. Besides, other inert matter present in the plant extract provides a 'buffer-like' effect which reduces toxicity. Since obesity and metabolic syndrome are complex conditions that require a multi-targeted approach, utilization of plant extract is a reliable and promising route.

1.5.1 Bioactive compounds

Metabolism refers to chemical reactions that occur in organisms to maintain life. The intermediate or end product of metabolism is called metabolites. Plants synthesize primary and secondary metabolites. Primary metabolites are compounds which are directly involved in the growth, development and reproduction of plants. They are

essential nutrients including carbohydrate, lipid, amino acid and lactic acid. Secondary metabolites on the other hand, are compounds which are not directly involved in the growth, development and reproduction of plants. Secondary metabolites function to protect the plant against harmful environments. They play important role in plant homeostasis by regulating the internal environment of the plant so that it can survive in extreme environments such as high temperature, and pathogen attack. Bioactive compounds are secondary metabolites (phytochemicals) present in plants which grant pharmacological or toxicological effect in humans or animals. Bioactive compounds are responsible for the therapeutic properties of plants. Various parts of medicinal plants including roots, barks, stems, fruits, flowers, branches, and calyces contain bioactive compounds (Surveswaran *et al.* 2007). Some classes of bioactive compounds which are commonly found in plants and utilized for medicinal purpose are glycosides, phenols, phenolic acids, terpenoids, flavonoids, iso-flavonoids, xanthones, saponins and anthocyanidins (M. M. Altaf *et al.* 2018).

For instance, thiacremonone, a bioactive compound found in garlic (*Allium sativum*) is reported to inhibit adipogenesis through downregulation of the adipogenic factors and therefore can be utilised for the treatment of obesity and its related disorders (E. J. Kim *et al.* 2012).

Besides that, Sasaki *et al.* (2007)also demonstrated the anti-obesity and anti-diabetic activity of cyanidin 3-glucoside, a bioactive compound present in abundance of fruits and vegetables including grapes. They reported that the anthocyanidin reduces blood glucose level and elevates insulin sensitivity in type 2 diabetic mice. Cyanidin 3-glucoside rich crude extract significantly normalized the hypertrophy of WAT by regulating the adiponectin level in HFD fed mice.

On the other hand, isoflavones including genistein, daidzen, and glycetin found in soybean (*Glycine max*) significantly lowered adiposity by regulating pro-inflammatory adipokines (IL-6, TNF-α, and resistin) level in rats fed with HFD. Treatment with isoflavones significantly improved insulin sensitivity and promoted lipid clearance in the liver (Jayarathne *et al.* 2017).

1.5.2 Current issues in drug discovery and development

Researchers are still struggling to find a cure for many global health issues including diabetes, degenerative diseases, HIV, obesity, and cancer using single compound drugs. Currently available treatments including synthetic compounds and natural drugs are insufficient to cater to the rapidly rising health issues. Hence, a reliable approach will be to return to mother nature as it has provided reliable answers for drug discovery in the past. Patwardhan and Mashelkar (2009) reported that 75% of anti-infective and 60% of anti-cancer drugs approved from 1981 to 2002 were naturally originated drugs. 34% of drugs approved by FDA between 1981 to 2010 were natural products (Harvey *et al.* 2015). WHO has also recognized, and created guidelines and standards for plant oriented medicine (Hosseinzadeh *et al.* 2015).

Although natural drugs are easier to reach the market than synthetic drugs, both drug discovery methods are expensive, require high capital investment and the success rate is low (Atanasov *et al.* 2015; S.F. Zhang *et al.* 2013). Since the public expectation for drug safety and efficacy is rising along with occurrence of multigenic diseases, the current ‘one drug fits all’ approach seems to be impractical. Thus, using a single drug to target one factor is certainly a bad idea. Instead, utilizing plant extracts without isolating a single compound may be a reliable approach as they provide multitarget

therapeutics (Gurnani *et al.* 2014). It is because often, presence of various bioactive compounds in plant extracts have proven to synergistically enhance therapeutic effect (Brusotti *et al.* 2014; Vermaak *et al.* 2011) increase bioavailability (Kowalska & Olejnik, 2016) and provide a 'buffer-like' effect (to reduce toxicity) (Pal & Shukla, 2003). Thus, using plant extracts for treatment of complex diseases is a promising alternative in terms of efficacy and toxicity.

1.5.3 *Phaleria macrocarpa, Hibiscus sabdariffa* and *Moringa oleifera*

Phaleria macrocarpa (*P. macrocarpa)), Hibiscus sabdariffa (H. sabdariffa)* and *Moringa oleifera (M. oleifera)* are three medicinal plants which are widely distributed especially in tropical countries. Table 1.6 shows the taxonomy of *M. oleifera* (Bhattacharya *et al.* 2017; Danish *et al.* 2011), *H. sabdariffa* (ITIS Standard Report Page: *Hibiscus*) and *P. macrocarpa* (Kavitha *et al.* 2018).

Table 1.6 Taxonomy of *P. macrocarpa, H. sabdariffa* and *M. oleifera*

	P. macrocarpa	***H.s sabdariffa***	***M. oleifera***
Kingdom	Plantea	Plantea	Plantea
Super division	Spermatophyta	Embryophyta	Spermatophyta
Division	Magnoliophyta	Magnoliphyta	Magnoliphyta
Class	Magnoliopsida	Magnoliopsida	Magnoliopsida
Order	Malvaves	Malvales	Capparales
Family	*Thymelaeaceae*	M*alvaceae*	*Moringaceae*
Genus	*Phaleria*	*Hibiscus*	*Moringa*
Species	*Macrocarpa*	*Sabdariffa*	*Oleifera*

1.5.3(a) *P. macrocarpa* pericarp

P. macrocarpa, commonly known as Mahkota Dewa (crown of god) is a plant originated from the Papua Island, Indonesia. It grows up to 6 m and is widely distributed in the tropical areas including Malaysia (Kusuma *et al.* 2011). *P. macrocarpa* has been conventionally used to treat various diseases including allergies, liver and heart related problems, diabetes mellitus, blood diseases, migraine, stroke