AN EVALUATION OF ANTIOXIDANT AND ANTIDIABETIC POTENTIAL OF CYNOMETRA CAULIFLORA (NAM-NAM, FABACEAE)

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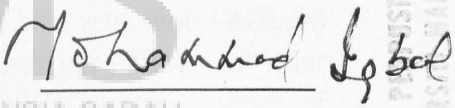

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ABSTRACT

Oxidative damage of biomolecules is implicated in the pathogenesis of various chronic diseases including diabetes. This has led to intensive investigation aimed at reducing the extent of such oxidative injury. *Cynometra cauliflora* or normally known as "Nam-nam" is a native of Malaysia, grown mainly in northern peninsular Malaysia and possesses many medicinal values in treating several diseases and for health care maintenance. However, antidiabetic and antioxidative potential of *C. cauliflora* have not been fully investigated. Therefore, the present study was aimed to evaluate the antioxidative, hypolipidemic and hypoglycemic potentials of C. *Cauliflora* extract against alloxan induced diabetes rats. Parts of *C.cauliflora* were used are; young leaves, matured leaves, stems and barks. The dry sample of young leaves, matured leaves, stems and barks from *C. cauliflora* were tested for phytochemical screening, total phenolic, flavonoids content and DPPH free radical scavenging activity. Phytochemical screening showed the presence of tannins, saponins and flavonoids in all parts of *C. cauliflora*. Terpenoids were found in all tested parts of the plant except in bark. The constituent of cardiac glycosides was present in all tested parts of the plant except in stem. Total phenolic content of young leaves was found to be 1831.47±1.03 mg/100g edible portion of dried sample. The total flavonoid content of young leaves was found to be a 33.63±0.25 mg/100g edible portion of dried sample. The radical scavenging activity was determined to be the following order: young leave>matured leave> stem> bark compared with ascorbic acid using as a standard. Based on the results of *in vitro* studies, matured leave was selected further for *in vivo* studies due to a high level of total phenolics and antioxidant capacity. Treatment of rats with alloxan resulted in a significant increase ($P<0.05$) level of blood glucose, total cholesterol and low density lipoprotein. On the other hand, oxidative stress was noticed in pancreatic tissue as evidenced by a significant decrease in glutathione level, catalase activity, and also significant increase in malondialdehyde when compared to normal saline control group. Pancreases were examined by hematoxylin and eosin staining. Additionally, serum biochemistry and oxidative stress markers were consistent with the pancreatic histopathological studies. Treatment of diabetic rats with *C. Cauliflora* extract significantly prevented these alterations and attenuated alloxan-induced oxidative stress. The results of the present study indicated that the hypolipidemic and hypoglycemic potentials of *C. Cauliflora* might be ascribable to its antioxidant and free radical scavenging properties. Thus, it concluded that *C. Cauliflora* may be helpful in the prevention of diabetic complications associated with oxidative stress.

ABSTRAK

PENILAIAN POTENSI ANTI-OKSIDAN DAN ANTI-DIABETIK TERHADAP CYNOMETRA CAULIFLORA (NAM-NAM, FABACEAE)

Kerosakan oksidatif biomolekul menyebabkan pelbagai patogenesis penyakit kronik termasuk diabetes. Cynometra Cauliflora atau biasanya dikenali sebagai "Nam-nam" adalah berasal dari Malaysia, ia mempunyai nilai perubatan dalam merawat beberapa penyakit dan untuk penjagaan kesihatan. Namun, potensi antidiabetik dan antioksida C.cauliflora tidak dikaji sepenuhnya. Oleh itu, objektif ini adalah untuk menilai potensi hipolipidemic dan hipoglisemik ekstrak C.cauliflora terhadap tikus yang diaruh oleh alloxan. Bahagian C.cauliflora digunakan adalah; daun muda, daun matang, batang dan kulit. Sampel C. cauliflora telah diuji untuk saringan fitokimia, jumlah kandungan fenolik, kandungan flavonoid dan aktiviti DPPH memerangkap radikal bebas. Ujian fitokimia menunjukkan kehadiran tanin, saponin dan flavonoid dijumpai dalam semua bahagian C.cauliflora yang di uji. Terpenoid telah di jumpai di semua bahagian tumbuhan yang di uji, kecuali bahagian kulit kayu. Komposisi glikosida kardium juga didapati pada semua bahagian tumbuhan yang di uji, kecuali dalam batang. Jumlah kandungan fenolik pada daun muda menunjukkan kadar 1831.47±1.03 mg/100g sampel kering pada setiap bahagian yang boleh dimakan. Jumlah kandungan flavonoid di dalam daun muda menunjukkan kadar 33.63±0.25 mg/100g sampel kering pada setiap bahagian yang boleh dimakan. aktiviti mengaut radical bebas menunjukkan susunan berikut daun muda >daun matang> batang > kulit kayu yang dibandingkan dengan asid askorbik sebagai penunjuk piawai. Keputusan analisis in vitro daun matang telah di pilih untuk menjalankan kajian in vivo berdasarkan jumlah fenolik dan aktiviti antioksidan. Tikus yang telah diaruh oleh alloxan menunjukkan perbezaan peningkatan yang ketara ($P<0.05$) di dalam aras kandungan glukosa dalam darah, kandungan kolesterol dan lipoprotein berketumpatan rendah. Selain itu, tekanan oksidatif wujud di dalam tisu pankreas sebagai membuktikan perbezaan penurunan yang ketara di dalam kadar glutathione, aktiviti pemangkin, dan juga perbezaan peningkatan pada kadar malondialdehyde apabila dibandingkan dengan kumpulan tikus yang normal. Tisu pankreas telah dikaji menggunakan teknik perwarnaan hematoxylin dan eosin . Serum biokimia dan penanda tekanan oksidatif adalah kosisten dengan kajian histopatologi tisu pankreas. Rawatan ke atas tikus diabetik dengan ekstrak C.cauliflora ketara mencegah dan melemahkan tekanan oksidatif yang di sebabkan aruhan alloxan. Keputusan analisis menunjukkan bahawa potensi hipolipidemik dan hipoglisemik dari C.cauliflora berkemungkinan memberi kesan berdasarkan kepada kadar antioksidan dan kadar pemerangkap radikal bebas. Justeru itu, keimpulan yang boleh dibuat adalah C.cauliflora berkemungkinan membantu dalam mencegah komplikasi diabetik yang berkait dengan tekanan oksidatif.

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ABBREVIATIONS

µg/ml	-	Microgram per milliliter
Abs	-	Absorbance
ALT	-	Alanine aminotransferase
AST	-	Aspartate aminotransferase
b.w.t	-	Body weight
C.cauliflora	-	*Cynometra cauliflora*
CAT	-	Catalase
df	-	Dilution factor
EC_{50}	-	Concentration giving 50% inhibition
$FeCl_3$	-	Iron chloride
g	-	gram
g/L	-	gram per liter
GSH	-	Reduced glutathione
IU/L	-	International units per liter
KCl	-	Potassium chloride
Kg	-	Kilogram
LPO	-	Lipid peroxidation
mg	-	milligram
mg/ml	-	milligram per millimetre
mmol/L	-	millimolar per liter
nm	-	nanometer
pH	-	Potential of hydrogen
PMS	-	Post mitochondrial supernatant
ROS	-	Reactive oxygen species
SEM	-	Standard error mean
w/v	-	Weight per volume
WHO	-	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background of study

Malaysia blessed to possess unique biological diversity and also to be rich in medicinal plants. Notwithstanding that, only about 300 species that are native to this country have been consumed, undergone clinical studies, and documented. According to the World Health Organization (WHO) 2013, in developing countries, about 80% of the human population in developing countries continue to use traditional medicine for their primary healthcare needs. This trend has captured the attention of many researchers to focus on the scientific evaluation of local medicinal plants.

Traditional medicinal plants were believed to used since time immemorial (Jantan, 2004). The knowledge of how to use medicinal plants was passed down from generation to generation (Naidoo, 2004). Many plants can produce a variety of bioactive compounds, also known as phytochemicals, and are good sources of saponins, tannins, flavonoids and polyphenols (Desai and Chavan, 2010). The antioxidant properties of medicinal plants have widely reported. Therefore, we find it worthwhile to plan a research to determine the efficiency of *C. cauliflora* to reduce diabetes, one of growing risk factors in our community.

C. cauliflora is an underutilized fruit usually grown in orchards or gardens around houses (Ikram *et al.*, 2009). *C. cauliflora*, also known as *nam-nam* was believed to be native to Malaysia (Mudiana and Darmayanti, 2010). This plant categorized in the genus *cynometra* in the kingdom *plantae*. *C.cauliflora*, a rare species, is a member of the *fabaceae* family and cultivated along the east coast of Peninsular Malaysia. The recorded height of *C. cauliflora* spans from 3 metres to 15 metres. This fruit found in the form of kidney-shaped pods(Mudiana and Darmayanti,2010). The *C.cauliflora* tree gets its local name because of the

shrubby fruit and brownish green colour of the fruit's skin. Old folks believe that this plant can treat high blood pressure and diabetes. In India, seeds of this genus were reported to be used in the treatment of skin diseases (Desai and Chavan, 2011).

Over the years, *C. cauliflora* has been increasingly consumed by our local community as it was believed to be beneficial for health due to its antioxidant and antidiabetic properties. According to a previous study, members of this genus contain high concentrations of polyphenols, tannins, flavonoids and saponins (Desai and Chavan, 2011). However, there are no studies reported using *C.cauliflora*. A high consumption of fresh fruits, vegetables, and herbal plants was associated with a decrease in risk of disease in mankind (Ames *et al.*, 1993). In recent years, there has been growing interest in establishing the therapeutic potential of medicines which are deemed to be natural, inexpensive and without adverse side effects (Gupta, 2011). On the other hand, the trading of natural products is highly lucrative in the international marketplace. Free radicals and reactive oxygen species (ROS) are believed to be responsible for the aging process, tissue damage and also a cause of serious diseases (Sultana *et al.*, 2012). In developing countries, many factors can contribute to the production of free radicals, such as smoking, sun exposure, pollution and exposure to toxic chemicals that can impact our health negatively. Secondary effects of biotic and abiotic stress result from the constant production of ROS, which includes free radicals. Usually, the production of ROS is a normal metabolic process, but if the production of ROS is uncontrolled, it can overwhelm the antioxidants in the human body which act as defences (Li *et al.*, 2011).

Oxidation is a process that damages cells, whereas antioxidants help prevent or delay oxidizing. Scientists believe that antioxidants can reduce the risk for chronic diseases (Tukaram *et al.*, 2011). Many fresh fruits, vegetables, and herbal plants have been found to contain antioxidants (Guan and Whiteman, 2003) and can categorize as primary sources of antioxidants. Antioxidant substances can terminate the chain reaction of free radical and ROS before they can cause any damage. Most antioxidant compounds derived from plant sources.

The main characteristic of antioxidants is their ability to trap free radicals (Tukaram *et al.*, 2011). In addition, antioxidant compounds can scavenge free radicals and give protection to cells by delaying the process of lipid peroxidation (Gulcin *et al.*, 2007).

The antioxidant defence system against free radicals in our body divided into four categories or lines of defence, based on their functions. The first line of defence is the preventive antioxidants that inhibit the formation of free radicals. Radical scavenging antioxidants represent the second line of defence, which inhibits chain initiation or taking steps to break down the chain of propagation reaction. The third line of defence presented by repair and *de novo* (afresh) antioxidants. An arrangement for the signal for production and reactions of free radicals occurs at the fourth line of defence that induces the formation and transport of the proper antioxidants to the right side (Noguchi *et al.*, 2000). Polyphenolic compounds found in plants, such as flavonoids, tannins, and phenolic acids seem to have high antioxidant properties, and these compounds are present in medicinal and dietary plants which help to prevent oxidative damage (Gupta, 2011). Several medicinal plants or extracts that contain antioxidant properties has been used to treat diabetes mellitus based on folklore medicine. Furthermore, several bioactive compounds that occur naturally in many plants have the potential of carrying out hypoglycemic activity. Therefore, antioxidants play a decisive role in order to treat diabetes.

Diabetes mellitus, commonly known as diabetes, is a metabolic disease characterised by hyperglycaemia (Varvarovska *et al.*, 2004) that reportedly affects 5% of the world population (Ndiaye *et al.*, 2008). According to survey result from National Health Morbidity Survey III (NHMS III), Zanariah *et al.*, 2008, the prevalence rate in Malaysia has risen much faster than expected. WHO predicted that in the year 2030, Malaysia would have a total of 2.48 million people with diabetes (zanariah et al., 2008).Diabetes can affect the eyes, kidney and nervous system, and it has also been attributed as a contributor to cardiovascular diseases (Pushparaj, 2004). Diabetes occurs when the pancreas does not produce enough insulin or does not properly use insulin that has been produced (WHO). Insulin is

a hormone that acts to transfer glucose in blood into cells as well as to lower the glucose level in blood. Diabetes also can cause development-related disorders, for example, heart disease that can lead to stroke, high blood pressure, blindness, kidney disease, nervous system diseases and dental diseases (American Diabetic Association, 2002). Recently, diabetes was listed as one of six leading disorders that can cause morbidity and death (Zeggwagh *et al.*, 2006). In contrast, research conducted for the past decade on scientific evaluation of medicinal plants show their potential to treat diabetes.

Hyperglycaemia causes many ROS, which in turn can cause membrane lipid peroxidation and degradation (Berbecaru-Iovan, 2009). Researchers now focus more on important properties of medicinal plants to discover new, safer drugs from natural sources. Therefore, the antidiabetic medications' main function is to maintain a normal glucose level in the blood (Berbecaru-Iovan, 2009). Diabetes is known to be associated with hypertension. The presence of both will increase the risk of cardiovascular diseases (Govindarajan *et al.*, 2006). In the past decade, medicinal plants or natural products have become a viable alternative solution to treat diabetes in mankind. Hence, the present study designed using various medicinal plant extracts plans to show their potential effect in a diabetic rat as an animal model.

In the past, animal models were frequently used in testing antihyperglycemic properties. Animal models were believed to resemble many features in humans (Badyal *et al.*, 2003).

1.2 Hypothesis

The hypotheses that can be derived from this study are:

a. C.cauliflora is expected to have a significant antidiabetic effect both *in vitro* and *in vivo*.

b. Antidiabetic properties of the *C. cauliflora* can be proven through antidiabetic studies and determination of phytochemical constituent.

1.3 Objectives

This investigation is to archive the following objectives:

a. To evaluate the phytochemical composition and antioxidative potential of *C. cauliflora*.

b. To determine the *in vivo* efficiency of *C. cauliflora* against alloxan-mediated changes in the levels of blood glucose, lipid profiles, and serum transaminases.

c. To assess the *in vivo* efficiency of *C. cauliflora* against the alloxan-mediated manifestation of oxidative stress in the pancreas by measuring malondialdehyde (MDA) concentration, the end product of lipid peroxidation and assessing total antioxidant power such as reduced glutathione and antioxidant enzymes.

d. To evaluate the *in vivo* efficiency of *C. cauliflora* against alloxan-mediated histopathological alterations in the pancreas.

1.4 Statement of problem

The number of people with diabetes is increasing, due to diet regime, environment, genetic and other factors. These facts contribute to more research demand in the diabetic field. *C.cauliflora* has been reported traditionally to be beneficial for diabetes treatment. However, there are no studies has done to support the claim. Hence, this study was aimed to explore scientifically antidiabetic and its antioxidant effect of *C.cauliflora* against alloxan monohydrate induced experimental rats. Oxidative stress is one of mechanism that involved in diabetes and leading to the development of complications. Free radical formation is caused by a chronic excess of glucose which leading to toxic effects on structure and impairment of organs function (Robertson, 2004). The present of antioxidant in plants and fruits can prevent the development of diabetes mellitus. Therefore, the activities of antioxidant in *C.cauliflora* also will be carried out.

1.5 Significance of the study

Extracts of *C.cauliflora* are expected to contain more biologically active components than other fresh fruits. Apart from containing antioxidants and vitamins, the non-nutritive components in the plant may exhibit potentially active

antioxidant phytochemicals that can combat oxidative stress. These findings will be value to consumers who are concerned about a healthy dietary intake and who consider natural sources as a great source of antioxidants to prevent the onset of various diseases. Diabetes is a chronic disease that affects half of the population in the world. Therefore, the present research is expected to provide a scientific basis for the use of this plant in folk medicine potential to maintained blood glucose level on diabetes through a reaction of antioxidants. . It will also support the agricultural industry in a positive way, leading to the economic well-being of farmers and fruit or plant traders alike. The growing of the *C.cauliflora on* a large scale will boost the national income through increased plantation and export.

CHAPTER 2

LITERATURE REVIEW

2.1 Botanical medicine

2.1.1 Botanical medicine in general

Ethnopharmacology is the scientific study of plants used medicinally by different ethnic groups. Many researchers get information about botanical medicine plants from village folks which have obtained traditional knowledge which was passed on for generations. Botanical medicine refers to the seeds, roots, leaves, bark and flowers of medicinal plants that are used as medicine. People all over the world have been using herbal plants since prehistoric times (Thovhogi, 2009). Despite the abundance of improvements in the development of synthetic drugs, most people in developing countries still depend on botanical herbal medicine due to the low costs and less toxicity that is associated with it (Okigbo and Mmeka, 2006).

In the last few decades, there has been an exponential growth in medicinal plant research in Malaysia, carried out by universities and research institutes which are mainly funded by the Malaysian government. Even though botanical herbal product screening programmes are robust, but it takes serious effort to initiate a medicinal plants discovery programme at the national level (Jantan, 2004). Most of the current researches on medicinal plants tend toward academic training, which is not concerned about development. However, some of the early findings from such researchers were not promised to be used in research in the development of new drugs. Jantan (2004) pointed that several problems pose major obstacles to the development of drug research in Malaysia.Such examples include the lack of effective research management and collaboration between research institutes, limitations in funding and the shortage of facilities to develop natural product-based drugs.

2.1.2 Secondary plant metabolites

Since centuries, many plant compounds play outstanding roles in medicinal and pharmacological activities, known as secondary plant metabolites (Thovhogi, 2009). Secondary plant metabolism occurs only in exceptionally differentiated cells and is not necessary for cells themselves. Ubiquitous macromolecules such as monosaccharides, polysaccharides, amino acids, protein, nucleic acids, and lipids are products of primary metabolism. Secondary plant metabolites with medicinal properties are only found in a few species of plants (Heinrich et al., 2004; Thovhogi, 2009).

2.2 *Cynometra cauliflora* (Nam-nam)

2.2.1 Background of *Cynometra cauliflora*

Cynometra cauliflora (*C.cauliflora*) belongs to the bean family, *Fabaceae* (formerly known as Leguminosae). This genus was established in 1741 by John D. Dwyer. According to Gao *et al.*, 2008. plant species from the *fabaceae* family are commonly used as traditional medicines and form the second largest family of medicinal plants. The *Fabaceae* family contains over 490 medicinal plants, which encompasses 20 genera and 31 species that are used in traditional medicines. *C.cauliflora* belongs to the kingdom *plantae* (Verheij & Coronel, 1991). *C.cauliflora*, meaning 'flower-stemmed', is generally known as *nam-nam* or *katak puru* in Kelantan, *Hima* in Thailand, and *kopi anjing* in Indonesia. It is believed to be native to Malaysia and also cultivated in Indonesia (Java Island) and India (Johannes Seidemann, 2005). According to Edward Balfour (1871), over 2 centuries ago, this plant was used to build bridges and buildings. Moreover, its roots, leaves and seeds (used for oil) are used as traditional medicine. The fruit of *C.cauliflora* resembles the shape of a kidney while the skin of this fruit is very rough. Due to its high acid content, *C.cauliflora* is sour when unripe, but the acid content in fruit decreases as it ripens. The flesh of the unripe fruit is dark green but turns yellowish-brown when ripe

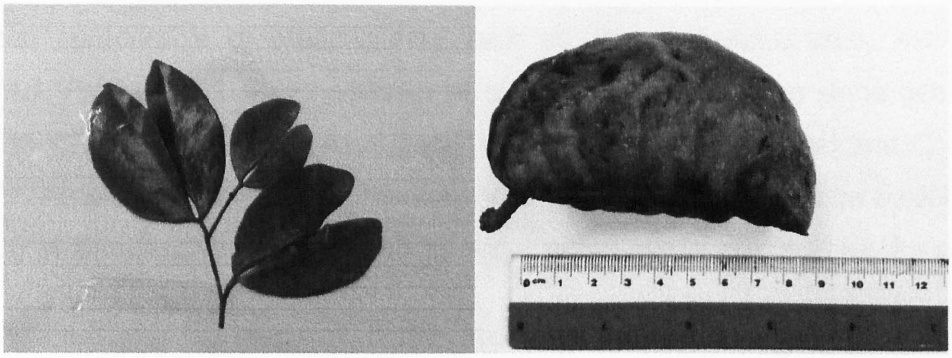

Figure 2.1: *Cynometra Cauliflora* leaves and Fruit.

2.2.2 Habitat and uses

C.cauliflora naturally grows well in wet tropical lowlands. Moreover, this plant also can grow in monsoon-type climates, which characterizes Malaysian weather. A malaysian climate is a suitable place for the growth of *C.cauliflora* growth, having an annual rainfall from 1500-2000 mm and a daily temperature of 22-35℃. *C.cauliflora* is also resistant to wind (Mudiana and Darmayanti, 2010). *C.cauliflora* plant has also become an attractive ornamental plant in home gardens and also grown as a bonsai (Verheij & Coronel, 1991). The yields of these plants are low. The plant was originally cultivated because of curiosity more than productivity (Verheij & Coronel, 1991). However, certain books point out that the roots, leaves and oil from the seed of *C.cauliflora* are used medicinally (Dwyer, 1758).

2.2.3 Phytochemical studies

C.cauliflora is an edible, underutilized fruit that is believed by old folks to treat or control diabetes mellitus. It is also believed to be able to treat gallstones and improved blood circulation. However, this fruit has not received a lot of attention from the scientific community, where there are not many scientific investigations conducted on this plant. From a previous study about a plant in the same genus and family with *C.cauliflora*, *C. iripa*, plants of this genus provide a rich source of saponins, tannins, flavonoids and polyphenols (Desai and Chavan, 2010). The intake of vegetables, fruits or medicine plants show positive results in reducing cardiovascular diseases and certain cancers (Ikram *et al.*, 2009).