EFFICACY AND SAFETY OF *ANNONA MURICATA* L. AS A PHARMACEUTICAL AGENT IN DIABETES: A SYSTEMATIC REVIEW

by

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LIST OF ABBREVIATIONS

AAI	Anti-atherogenic index
A1C	Hemoglobin A1c test
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BuOH	Butanol
CAM	Complementary and alternative medicine
CAT	Catalase
CH_2Cl_2	Dichloromethane
DMDP	2,5-dihydroxymethyl-3,4,-dihydroxypyrrolidine
DMJ	Deoxymannonojirmycin
DNJ	Deoxynojirmycin
DPP-4	Dipeptidyl peptidase-4
EtOAc	Ethyl acetate
EtOH	Ethanol
FOXO1	Forkhead box protein O1
FRAP	Ferric reducing antioxidant power
GC-MS	Gas chromatography-mass spectrometry
GDM	Gestational diabetes mellitus
GIP	Glucose dependent insulinotropic peptide

GLP-1	Glucagon-like peptide- 1
GSH	Glutathione
GSH-Px	Glutathione peroxidase
HSV-1	Herpes simplex virus-1
H2O	Aqueous fraction
Hex	Hexane fraction
HPLC	High Performance Liquid Chromatography
HDL	High density lipoprotein
IDDM	Insulin dependent diabetes mellitus
IFN-y	Interferon gamma
IL-10	Interleukin-10
INOS	Inducible nitric oxide synthase
I.P	Intraperitoneal
Kcal	kilogram calorie
LC50	Concentration required for reducing the 50% rate of reaction
LD50	Median lethal dose
LDL	Low density lipoprotein
LUHMES	Lund human mesencephalic cell line
MCF-7	Michigan Cancer Foundation-7
MDA	Malondialdehyde
Mg / ml	Milligram per milliliter
µg mL-1	Microgram per mililitre
MODY	Maturity onset diabetes of the young

NPH	Neutral protamine hagedron
P.o	per oral
ORAC	The method of oxygen radical absorbance capacity
ROS	Reactive oxygen species
SOD	Superoxide dismutase
STZ	Streptozotocin
TBARS	Thiobarbituric acid reactive substances
TC	Total cholesterol
T1D	Type 1 diabetes
TG	Triglyceride
TZDs	Thiazolidinedione
VLDL	Very low density lipoprotein

KEBERKESANAN DAN KESELAMATAN *ANNONA MURICATA* L. SEBAGAI AGEN FARMASEUTIKAL UNTUK DIABETES: SATU ULASAN SISTEMATIK

ABSTRAK

Diabetes mellitus merupakan satu sindrom gangguan metabolik disebabkan oleh hiperglikemia kronik kerana ketiadaan insulin atau pengeluaran insulin yang tidak mencukupi. Tumbuhan ubat-ubatan telah digunakan secara meluas di seluruh dunia untuk merawat diabetes. *Annona muricata* L. adalah tumbuhan daripada keluarga Annonaceae dan diketahui mempunyai kesan antihiperglisemia. Dalam beberapa dekad yang lalu, banyak penyelidikan saintifik telah dijalankan untuk mengkaji kesan antidiabetik *A. muricata* dan aspek keselamatan penggunaannya. Walaubagaimanapun, tiada sorotan kajian yang sistematik berasaskan bukti mengenai topik ini dapat diperoleh. Oleh itu, kajian ini telah dijalankan untuk menyediakan rumusan kajian terkini secara komprehensif mengenai aktiviti antidiabetik, mekanisme tindakan antidiabetik yang mungkin dan kesan-kesan toksik *A. muricata*. Enam pangkalan data dalam talian iaitu PubMed, Web of Science, Scopus, ScienceDirect, Google scholar, SpringerLink telah digunakan untuk mencari secara sistematik artikel-artikel berkaitan sepanjang tempoh 2002 hingga April 2018. Istilah carian yang digunakan ialah *A. muricata*/ soursop/ graviola/ guanabana dan diabetes, antihyperglycemia, glukosa dan toksisiti/ keselamatan. Jumlah akhir artikel yang dipertimbangkan dalam kajian ini ialah dua puluh empat. Berdasarkan sorotan kajian, ekstrak daripada pelbagai bahagian berbeza pada *A. muricata*, terutamanya daun menunjukkan kesan signifikan terhadap parameter antidiabetik yang merangkumi pengurangan glukosa darah, rangsangan terhadap rembesan insulin dan pemulihan berat

badan dan profil lipid. Kajian selanjutnya menjelaskan mekanisma antidiabetik yang mungkin bagi *A. muricata* seperti penambah baikan enzim antioksidan, pengurangan peroksidasi lipid, kesan perlindungan terhadap pankreas dan hati serta perencatan aktiviti enzim-enzim yang bertanggungjawab dalam metabolisma karbohidrat. Berhubung dengan aspek keselamatan *A. muricata*, sorotan kajian menunjukan penemuan yang bercanggah. Kajian *in vivo* tidak menunjukan sebarang kesan toksik setelah pengambilan ekstrak buah dan biji pada model tikus. Sebaliknya, kajian *in vitro* melaporkan kesan neurotoksik dan neurodegeneratif *A. muricata* dan komponen asetogenin and alkaloids. Kesimpulannya, *A. muricata* menunjukkan potensi terapeutik dalam rawatan penyakit diabetes. Walaubagaimanapun, kajian lanjut diperlukan untuk mengenal pasti sebatian aktif yang bertanggungjawab terhadap kesan antidiabetik dan untuk menentukan nilai ambang sebatian-sebatian ini sebelum kajian klinikal dapat dijalankan. Kajian keselamatan secara *in vivo* terhadap neurotoksin-neurotoksin *A. muricata* juga diperlukan untuk mengesahkan kesan toksik mereka terhadap manusia.

EFFICACY AND SAFETY OF *ANNONA MURICATA* L. AS A PHARMACEUTICAL AGENT IN DIABETES: A SYSTEMATIC REVIEW

ABSTRACT

Diabetes is a metabolic disorder characterized by chronic hyperglycemia due to the absence or insufficient production of insulin. The use of medicinal plants in the management of diabetes is globally spread. *Annona muricata* L. is a plant of Annonaceae family and known to have antihyperglycemic effect. During the past few decades, numerous scientific investigations have been conducted on the antidiabetic effects of *A. muricata* and its safety effects. However, no comprehensive evidence-based systematic review regarding these topics is available. Hence, this review was aimed to summarize up-to-date and comprehensive studies on the antidiabetic activity, the possible mechanism of antidiabetic action and the possible toxic effects of *A. muricata*. Six online databases which were PubMed, Web of Science, Scopus, ScienceDirect, Google scholar, SpringerLink were used to systematically search for the related articles over the period of 2002 till April 2018. The search terms used were *A. muricata*/ soursop/ graviola/ guanabana and diabetes, antihyperglycemia, glucose and toxicity/ safety. The final articles included in this review were twenty-four. Different parts of the plant extracts, particularly leaves showed significant effect on the antidiabetic parameters which include reduction of blood glucose, stimulation of insulin secretion and restoration of body weight and lipid profile. Further studies have elucidated the possible mechanisms of antidiabetic effect which includes improvement in antioxidant enzymes, reduction of lipid peroxidation, pancreas protective

and hepatoprotective effects, as well as inhibition of carbohydrate metabolizing enzymes. On the hand, safety studies of *A. muricata* reported mixed findings. *In vivo* studies showed no toxic effect upon ingestion of pulp and seed extracts in rat models. On contrary, several *in vitro* studies have reported neurotoxicity and neurodegenerative effects of *A. muricata* and its isolated acetogenins and alkaloids. As a conclusion, *A. muricata* has a promising therapeutic effect against diabetes. However, further investigations are required to identify the active principles responsible for the antidiabetic effect and to determine the threshold value of these compounds at which this effect is caused before any clinical trial could be conducted. *In vivo* toxicity study on the effect of neurotoxins of *A. muricata* is also needed to verify their toxic effects on human.

CHAPTER 1

INTRODUCTION

1.1 Background Diabetes mellitus is a group of metabolic disorders characterized by persistence hyperglycemia due to the abnormalities in insulin secretion and/or insulin function (Alotaibi et al., 2017, Association, 2010). Symptoms of hyperglycemia are polyuria, polydipsia, polyphagia, weight loss and blurred vision. Sometimes with chronic hyperglycemia impairment of growth and susceptibility to different infections may also be present. Chronic diabetes may cause serious long term complications such as retinopathy, nephropathy, peripheral neuropathy, foot ulcers and diabetic foot (Association, 2010). These complications lead to certain organs damage, such as eyes, kidneys, nerves, heart, and blood vessels.

The prevalence of diabetes mellitus has drawn global attention as the total number of adult people with diabetes in 2017 was approximately 425 million people and the number is estimated to be 629 million in 2045 (International Diabetes Federation, 2017). Globally, there are 10 countries accounting for 60 % of total adult diabetic patients with four of the top ten countries are Asian countries which are China, India, Indonesia, and Pakistan. According to IDF statistics, in 2017 the total number of deaths in adults due to diabetes was about 4 million people; approximately half of them are under age of 60.

Controlling diabetes involves dietary management, life style modification, insulin therapy and oral hypoglycemic drugs as well as complementary and alternative medicine (CAM) therapies (Bharti et al., 2014). The widespread of CAM namely medicinal plant in both

developed and developing countries is attributable to its affordability, accessibility and efficacy (Dayeef et al., 2013). Despite the development of new drugs and their validation by scientific criteria, the research continues to investigate antidiabetic potential of various raw material or isolated natural products that likely to pose no adverse effects. Additionally, it is believed that an effective natural adjuvant therapy would be blindingly beneficial to diminish diabetic complications and augment the quality of life for diabetic patients.

The popular fruit tree of *Annona muricata* L. (Annonaceae) commonly known as graviola or soursop is one of the medicinal plant that has been scrutinized intensively for its promising pharmacological effects. It is commercially planted in Central and South America, tropical and subtropical parts of the world (Adewole & Caxton-Martins, 2006). *A. muricata* is an evergreen, terrestrial, erect tree reaching 5–8 m in height and features an open, roundish canopy with large, glossy, dark green leaves. The edible fruits of the tree are large, heart-shaped and green in color, and the diameter varies between 15 and 20 cm. All parts of the graviola tree are used in natural medicine, including bark, leaves, roots, fruit, and seeds. According to (de Souza et al., 2009), different parts of the tree have different medicinal values. Recently, many ethnomedicinal studies have indicated that *A. muricata* has become widely used for its hypoglycemia effect (Karou et al., 2011, Ezuruike & Prieto, 2014).

1.2 Problem statement

During the past few decades, numerous folk medicinal and scientific investigations on the antidiabetic effects of *A. muricata* have been reported. However, no comprehensive evidence-based review is available.

1.3 Significance of the study

Annona muricata is used widely in folkloric medicine to treat numerous medical disorders; one of them is diabetes, one of the fastest growing chronic diseases globally. The effect of *A. muricata* on diabetic parameters has been proved through *in vivo* and *in vitro* approaches. Different parts of the plant showed direct improvement on the blood glucose level, pancreas morphology, serum insulin concentration and lipid profile. In addition to its efficacy on diabetes, the safety and toxicity of the plant has also been considered and several studies have been conducted on this regard. Even though many scientific studies have been conducted and reported, there is no comprehensive evidence-based review available regarding these topics. Hence, this review was aimed to summarize up-to-date and comprehensive studies on the efficacy and safety of *A. muricata* as an antidiabetic agent. This review could be a reference for both, the researchers and the pharmaceutical industries into developing and commercializing a new plant-based drug for diabetes management.

1.4 Objectives of the study

The main objective of the present study is to systematically evaluate the literatures on the effects of *A. muricata* extracts in managing diabetes and document potential toxic effects. The specific objectives of this study are:

1. To summarize an up-to-date and comprehensive studies on the antidiabetic activity of *A. muricata.*
2. To summarize the possible mechanism of antidiabetic action of the parts and active principles of *A. muricata.*
3. To summarize the possible toxic effects of *A. muricata.*

CHAPTER 2

LITERATURE REVIEW

2.1 Definition and classification of diabetes

The term "diabetes" is a Greek word which was first used by Aretaeous of capadocia which literally means "to pass through" to describe the condition that is characterized by high sugar level in blood and urine, hunger and thirst. While "mellitus" is (a latin-greek word that means "honey") was introduced by the English physician John Rollo to explain the conditions among other polyuric diseases, in which glycosuria does not occur (Alexiou & Demopoulos, 2010).

According to American diabetes association diabetes mellitus is classified into type 1 diabetes, type 2 diabetes, gestational diabetes and other types of diabetes. Type 1 diabetes also known as juvenile or insulin dependent diabetes mellitus (IDDM) or immune mediated type 1 diabetes, is characterized by deficiency of insulin secretion from pancreas due to the loss or destruction of pancreatic beta cells (Organization, 1999). Insulin is an essential hormone for regulation of the carbohydrate, fat and protein metabolisms in the body. It is also promoting the intake of glucose from the blood into liver, fat and skeletal muscle cells (Sonksen & Sonksen, 2000). Destruction of beta-cells is usually due to autoimmune reaction, multiple genetic predispositions and also environmental factors that are still not completely understood. Whereas in some cases may have no known etiology and no evidence of autoimmunity is present, this type is known as idiopathic type 1 diabetes. Type 1 diabetes patient requires insulin therapy replacement in order to survive (Association, 2010).

Type 2 diabetes is characterized by insulin resistance and/or insulin deficiency. In some cases, the insulin level appears to be normal or elevated with high insulin resistant in the body. Obesity is one of the common reasons of insulin resistance. Therefore, changing in lifestyle and diet as well as exercise to reduce the body weight are the effective ways of managing type 2 diabetes. Available treatment approaches include oral anti hyperglycemic agents, e.g. biguanides, sulphanylureas and dipeptidyl peptidase 4 inhibitors (Turner et al., 1999).

Gestational diabetes mellitus (GDM) is characterized by any glucose intolerance that is diagnosed first during the pregnancy. It is reversible in most cases and resolves after the delivery. During the first and first half of second trimester of pregnancy, the level of blood glucose is usually low. Hence, any elevation in fasting blood glucose and/or postprandial plasma glucose level indicates the presence of gestational diabetes. It is necessary to start screening or do risk assessment of gestational diabetes from the first prenatal visit as an earlier therapy to lower the risk of mortality and morbidity (Organization, 1999).

Other types of diabetes mellitus may involve several types of diabetes which are less common. For example, genetic defect of beta-cell function which is characterized by inherited insulin secretion impairment and insulin action deficiency. This condition mostly reported at an early age before 25 years and is referred as maturity onset diabetes of the young (MODY). Other type of diabetes is endocrinopathies which happened due to excessive intake of some specific hormones, like growth hormone, cortisol and glucagon. These hormones may antagonize the action of insulin. In addition to that, several drugs and certain viral infections have been associated with beta-cell destruction that lead to diabetes (Association, 2010).

2.2 Management of diabetes mellitus

Management of diabetes involves diet, oral anti hyperglycemic drugs and insulin therapy. According to (DeFronzo et al., 2015), diabetes mellitus is a chronic metabolic disorder that requires continuous medical care with patient personal management education in order to achieve glycemic control and prevent other diabetic complications.

2.2.1 Diet

The aim of dietary management is to achieve glycemic control and optimize the patient's sense of clinical well-being by reducing the total amount of glucose intake (Bharti et al., 2014). As a result, the patient's body weight will reduce, prevent weight gain and improve insulin resistance. There are two types of diet used widely, which are low energy weight reducing diets and weight maintenance diets. The main target of low energy weight reducing diet is to maintain about 0.5 kg weight loss per week by causing 500 kcal daily deficits. As it is an intensive diet weight reduction, the patient must be under proper care to avoid any complications resulting from the loss of body essential nutrients, vitamins and minerals.

Weight maintenance diet is especially for those with normal body mass index. This diet is characterized by high carbohydrate and less fat intake with certain attention to the type of fats (Bharti et al., 2014).

2.2.2 Insulin therapy

The primary target of insulin treatment is to replace the insulin through exogenous insulin injection. There are many types of purified insulin preparation available commercially. The most common preparation is administered via subcutaneous injections. These preparations

are classified according to their duration of action. Short acting or Rapid Acting Insulin regular, (R) insulin acts rapidly as its action begins in about 30 minutes after injection. Therefore, it is recommended to be administered 30 minutes before meal. The duration of action is about 5 to 8 hours. This class is effective in managing high postprandial glucose or acute hyperglycemia of hospitalized patients in perioperative period. Examples of rapid acting insulin are lispro, aspart and glulisine insulin. The second class is intermediate acting insulin. Neutral protamine hagedron, (NPH) insulin is intermediate acting insulin which is administered twice daily due to its intermediate duration of action, it is known as the most cost-effective insulin. Lastly is the long acting insulin. Glargine insulin is a long acting or basal insulin which is administered once daily since its lasts longer than 24 hours. The cost of glargine insulin, however is higher and it causes discomfort at site of injection (Inzucchi & Sherwin, 2011).

2.2.3 Oral antidiabetic drugs

Oral pharmacological treatment is required in the management of type 2 diabetes. In the middle of 20th century, several anti-diabetic drugs have been approved by the Food and Drug Administration (FDA). Between 1950s and 1960s, the first generation of sulfonylurea drug, tolbutamide was approved, followed by other first-generation of sulfonylureas, chlorpropamide, acetohexamide, and tolazamide. In the 1959, a biguanide, phenformin, was approved (Kennedy et al., 1988), before it was recalled as an "imminent hazard" in 1977 and found to be associated with fatal lactic acidosis (Misbin, 1977). The second biguanide, metformin was approved by FDA in 1995, followed by glimepiride in 1996, acarbose the first glucosidase inhibitor and repaglinide, a nonsulfonylurea insulin secretagogue in 1997. Then, in 1999 several more anti-diabetic drugs were approve, such as thiazolidinedione,

rosiglitazone and pioglitazone and the second glucosidase inhibitor, miglitol (Wysowski et al., 2003).

Biguanides:

The only biguanide approved to be safe and effective against type 2 diabetes is metformin. It acts by lowering hepatic glucose output and reducing fasting blood glucose. It has been reported that metformin can reduce hemoglobin A1c test (A1C) level by 1.5 percent when used alone as monotherapy (DeFronzo et al., 1995, Bailey & Turner, 1996) and it does not cause hypoglycemia. The common side effect of metformin is gastro intestinal disturbances. It has been claimed that metformin has effect on cardio vascular disease outcomes (Group, 1998). The use of metformin in renal failure patients is contraindicated as metformin can induce risk of lactic acidosis.

Sulfonylureas:

Sulfonylureas can treat hyperglycemia by stimulating insulin secretion from the pancreas. Similar to metformin, it lowers A1C levels by 1.5 percentage points. The common side effect of sulfonylureas is hypoglycemia; sever hypoglycemia is most frequently developed in elderly. Chlorpropamide and glibenclamide which are also known as glyburide are sulfonylureas known to be associated with higher risk of hypoglycemia than the other second-generation sulfonylureas like gliclazide, glipizide and glimepiride. Monotherapy of sulfonylureas shows fast onset of the glucose reduction effect. Sulfonylurea therapy was associated with the increase risk of cardiovascular disease mortality (Goldner et al., 1971).

Dipeptidyl peptidase four inhibitors:

Dipeptidyl peptidase four inhibitors are small molecules that improve the effect of GLP-1 and glucose dependent insulinotropic peptide (GIP), mainly incretins in the intestine. DPP-4 inhibitors stimulate glucose-mediated insulin secretion and suppress glucagon secretion. DPP-4 inhibitors are approved recently by food and drug administration (FDA) in october 2006, the first oral DPP-4 inhibitor was approved is sitagliptin, to use as monotherapy, or in combination with metformin or thiazolidinedione, while vildagliptin was approved in 2008. DPP-4 inhibitors can reduce A1C by 0.6 to 0.9 percentage points (Raz et al., 2006). When DPP-4 inhibitors used as monotherapy, no hypoglycemia can be developed. But, it has been reported that there is a possibility to interfere with immune function and induce risk of upper respiratory tract infections (Richter et al., 2008).

Thiazolidinedione:

Thiazolidinedione (TZDs or glitazone) is an insulin sensitizer which increase the sensitivity of muscles, fat and liver to endogenous and exogenous insulin (Yki-Järvinen, 2004). When thiazolidinedione used as monotherapy it can reduce the A1C by 0.5 to 1.4 percentage point. The common side effects of thiazolidinedione is peripheral edema because of fluid retention, congestive heart failure, weight gain and induce incidence of bone fractures mostly in women (Home et al., 2007).

Alpha glucosidase inhibitors:

Alpha glucosidase inhibitors act by delaying the digestion of polysaccharides in the small intestine and hence, reducing postprandial blood glucose. These inhibitors can decrease A1C by 0.5 to 0.8 percentage point. Clinical-trails suggested the use of alpha glucosidase inhibitors is not associated with the risk of hypoglycemia, malabsorption and weight loss.

The common side effects of this drug are production of gases and gastrointestinal discomfort due to the high delivery of carbohydrates to the colon. And because of this unwanted side effect, about 25–45% of patients who have stopped their using of alpha glucosidase inhibitors (Van de Laar et al., 2005, Chiasson et al., 2003). In the clinical, acarbose also showed a potential benefit in reducing the risk of cardio vascular disease and its complications in high risk diabetic patients.

Meglitinides:

Also known as glinides, these drugs stimulates insulin secretion, similar to sulfonylureas, but with less duration of circulatory half-life. Repaglinide has significant effect on A1C levels when used as monotherapy, the effect which is comparable to metformin or sulfonylureas. Whereas nateglinide is less effective than repaglinide in efficacy on A1C level (Rosenstock et al., 2004). Unlike sulfonylureas, there is low risk of hypoglycemia when using glinides as a monotherapy, but weight gain effect is still observed (Bharti et al., 2014).

2.3 Annona muricata L.

In the pharmaceutical field, large variety of plants and natural products has been studied thoroughly with the aim to find the most effective drug with fewer side effects. Those plants are a rich source of active phtyochemicals that hold potential to be a cure against various ailments. One of those plants is *Annona muricata*. *A. muricata* is a family member of Annonaceae and locally distinguished as guanabana, soursoap or graviola. In this

section, the botanical, distribution, ethnomedicinal uses and phytochemistry of this plant will be described briefly.

2.3.1 Botanical description and distribution

A. muricata is an evergreen upright tree, which can reach up to 5-7 meters in height. The leaves of this plant appear to be shiny and smooth with pointy ends and their lengths are between 2 to 15 centimeters. The flowers are huge and lone and yellowish to green in shading. The fruit is oval-shaped with 20 centimeters in diameter and is secured with scattered spine-like structures. The pulp is fleshy white and juicy fibrous, with a unique sweet and sour flavor. A fruit may contain up to at least 200 seeds. *A. muricata* is a widespread tree and local to tropical America, but currently is planted worldwide. *A. muricata* is distributed for the most part of tropical and sub-tropical regions, including South and North America, Australia, India, Nigeria and Malaysia (Moghadamtousi et al., 2015).

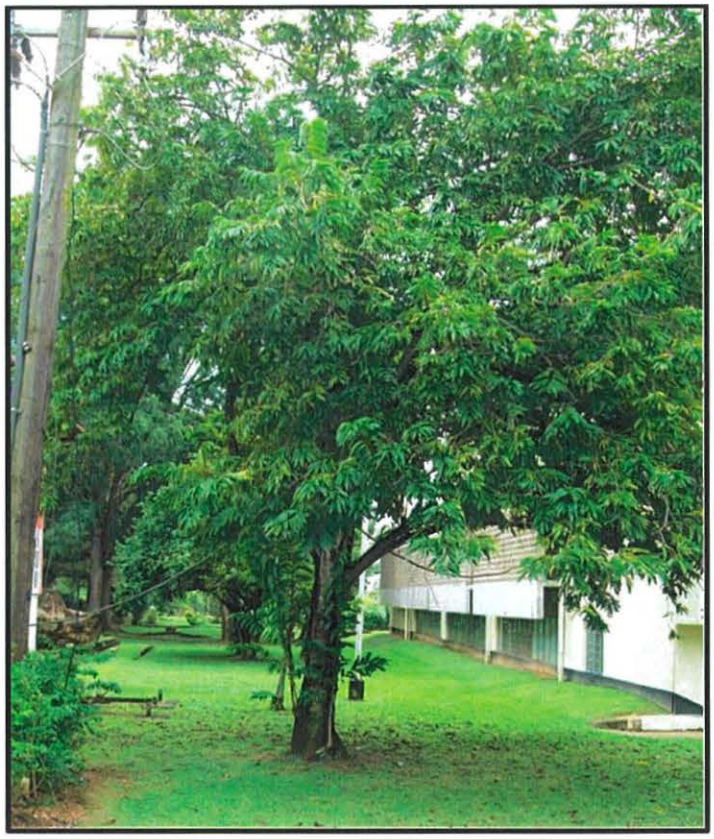

Figure 2.1 Soursop tree, photograph cited from (Badrie & Schauss, 2010)

Figure 2.2 Soursop fruit, photograph cited from (Moghadamtousi et al., 2015)

Figure 2.3 Soursop leaves, photograph cited from (Moghadamtousi et al., 2015)

Figure 2.4 Soursop flower, photograph cited from (Moghadamtousi et al., 2015)

2.3.2 Ethnomedicinal use

All parts of *A. muricata* tree are employed in traditional medicine to treat numerous medical disorders and diseases. As per folkloric claims, this plant has high efficacy against cancer and parasitic infections. The fruits are used to treat many conditions like inflammatory diseases, diarrhea, fever, malaria, parasites and used to stimulate lactation in women after delivery. Leaves are effective against headache, sleep disorder and diabetes. Some studies reported that ingestion of leaf's decoction exhibited anti-rheumatic and neuralgic effects, while the cooked leaves are used externally to heal abscesses and rheumatism (Mishra et al., 2013, De Sousa et al., 2010). 1mg/kg of methanolic extract of *Annona muricata* showed human antiviral activity against herpes simplex virus-1 (HSV-1) (Gajalakshmi et al., 2012). Furthermore, aqueous extract of leaves had been reported to have cytotoxic effect against women breast cancer and ethanolic extract of Annona leaves demonstrated significant cytotoxic activity against the Michigan Cancer Foundation-7 (MCF-7) cell line with the respective IC_{50} values of 14.678ug/ml and 88.788ug/ml on 24 and 48 hours (Endrini et al., 2015). Interestingly, ethanolic extract of leaves were also found to be effective against five different gram positive as well as gram negative bacteria species in agar disc diffusion method (Chithra et al., 2016). The phytochemical analysis and total phenolic compounds of methanolic and aqueous extract of *Annona muricata* for the DNA protective and free radical scavenging effects using high performance liquid chromatography (HPLC) method had revealed significant DNA protective and free radical scavenging activities of both extracts with methanolic extract of *Annona muricata* L. exhibited greater effect than aqueous extract (George et al., 2015). In Malaysia, the combination of crushed leaf of *A. muricata* with *A. squamosa* and *Hibiscus rosa-sinensis* is applied on the head to prevent fainting (Ong & Norzalina, 1999).

2.3.3 Phytochemistry

Several methods have been used to identify phytochemical constituents of different parts of *A. muricata*. As a result, various phytoconstituents have been reported including alkaloids (Yang et al., 2015), annonaceous acetogenin compounds (Rupprecht et al., 1990), cyclopeptides (Kossouoh et al., 2007), megastigmanes (Matsushige et al., 2012), flavonol triglycosides (Nawwar et al., 2012) and phenolics (Jiménez et al., 2014). The fruit of *Annona muricata* contains high concentration of major minerals like potassium, calcium, sodium, copper, iron and magnesium. Thus, this fruit can be considered as a rich source of minerals and consuming this fruit on a regular basis can supply essential nutrients needed for the body (Badrie & Schauss, 2010, Gajalakshmi et al., 2012).

CHAPTER 3

METHODOLOGY

3.1 Search strategy

Databases used for automated search were, PubMed, Web of Science, Scopus, Science Direct, Google scholar, Springer Link from 2002 till April 2018. The following keywords were used to retrieve all the included studies; *Annona muricata*/ Soursop/ Graviola/ Guanabana and diabetes, antihyperglycemia, glucose and toxicity/ safety. Additionally, manual search was carried out from the reference list of included articles for additional data or forward citation (Figure 3.1).

3.2 Study selection and inclusion/ exclusion criteria

The title and abstract of each article was assessed and the duplicated articles were removed. Any irrelevant papers were excluded. The remaining articles were further assessed to determine their compatibility with the inclusion criteria (refer to Figure 3.1). Articles in languages other than English, review articles, conference proceedings, commentaries, abstract in symposium and congresses were excluded.

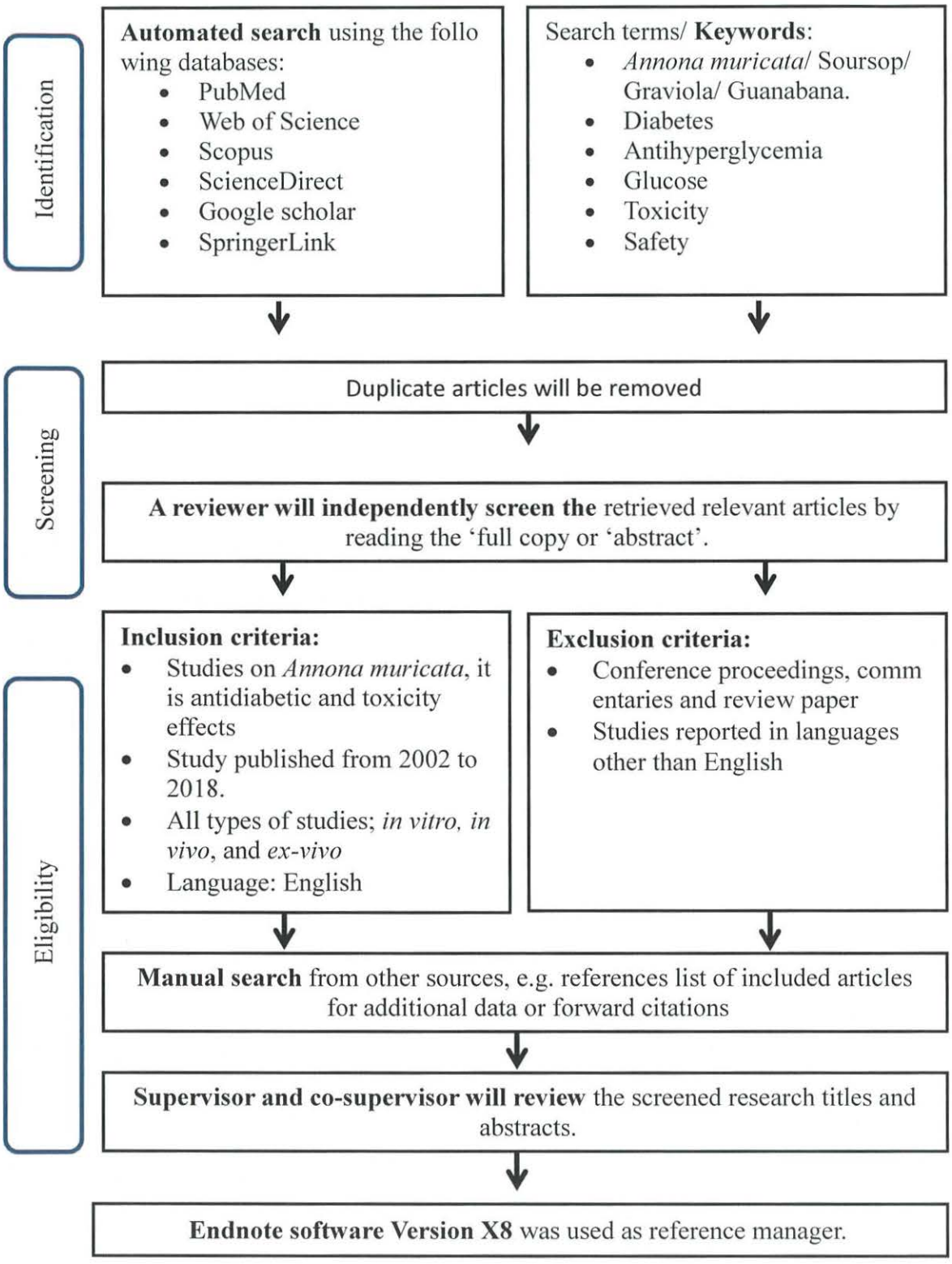

Figure 3.1 Trend of screening and choosing articles

CHAPTER 4

RESULT AND DISCUSSION

4.1 Literature search

The literature searched yielded a total of 167 records (from the year 2002 to April 2018). The following are the numbers of articles extracted from the respective databases; Google scholar (n=68), ScienceDirect (n=35), PubMed (n=25), Web of Science (n=19), Scopus (n=10) and SpringerLink (n=10). After removing the duplicate articles, 132 articles remained, of which 91 were excluded based on title and abstract. Forty one articles were further assessed by reading the full text, and a total of 17 articles were excluded; eight articles were conference proceeding and review articles, five were ethnobotanical studies, 3 articles were considered not valid for this study and one study was presented in language other than English, e.g.(Suastuti et al., 2015). We were unable to obtain any relevant study through manual search of the references of included papers or from any other data sources. The final number of studies that met the inclusion criteria in the present review was 24 studies. The applied search strategy was summarized in Figure 4.1.

As depicted in Figure 4.2A, most of the included studies were conducted by research groups from Nigeria which accounted for 11 studies, followed by France (4 studies), then Brazil and Indonesia (3 studies each). Studies conducted by researchers from different countries were also observed. The number of study is highly related to the geographical distribution of *Annona muricata* as the local researchers will give more focus to the native plants.

The highest number of articles published was in 2015 and 2017 which comprised 4 studies for each year, followed by 2008, 3 studies, and 2 studies in both 2010 and 2013 (Figure 4.2B).

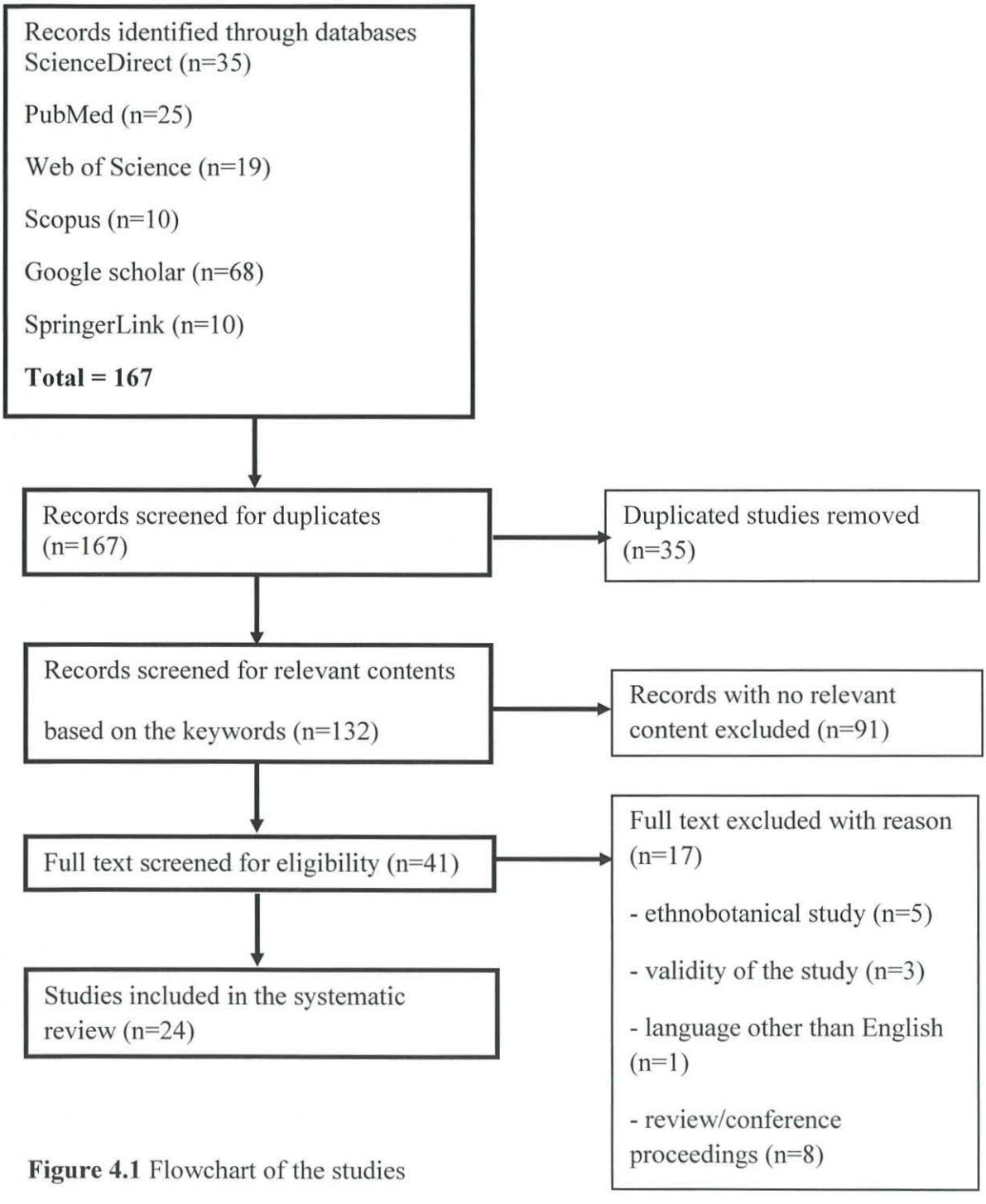

Figure 4.1 Flowchart of the studies

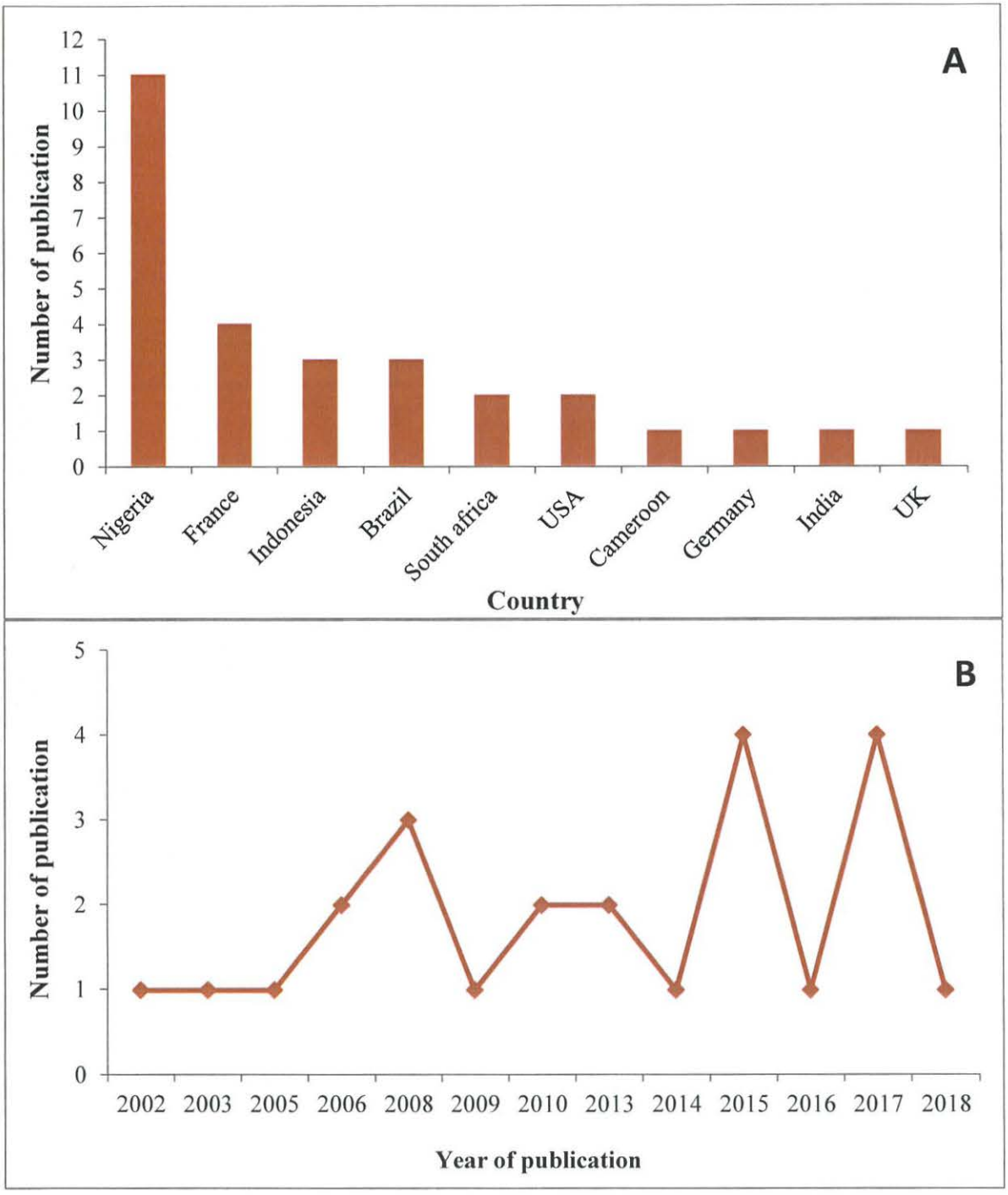

Figure 4.2 Distribution of the selected manuscript for the systematic review according to (A) country (B) year of publication

4.2 Classification of the antidiabetic study

From a total of 24 articles included in the present review, 15 articles discussing the efficacy of *Annona muricata* as an antidiabetic agent were identified. Those articles were classified into five groups based on the applied study models which were clinical, animal *in vivo, in vitro,* combination of *in vivo* and *in vitro* and computational studies (Table 4.1). To date, no clinical study had been conducted on this plant. On the other hand, there were 10 *in vivo* studies reported using animal models, 3 *in vitro* studies, one study combining both *in vitro/in vivo* models and lastly one computational or *in silico* study. These classified studies have been summarized in Table 4.2, 4.3 and 4.4. The other 9 studies included in our review were on the safety and toxicity of *Annona muricata*.

Table 4.1 Classification of studies on the antidiabetic activities of *A. muricata*

Study model	**References**
Clinical study (n=0)	
Animal *in vivo* study (n=10)	(Adeyemi et al., 2009b, Florence et al., 2014, Adeyemi et al., 2009a, Agbai et al., 2015, Hasanah et al., 2017, Ahalya et al., 2014, Adeyemi et al., 2010, Adewole, 2010, Adewole & Caxton-Martins, 2006, Adewole & Ojewole, 2009)
In vitro study (n=3)	(Berawi et al., 2017, Adefegha et al., 2015, Justino et al., 2018)
In vivo and *in vitro* (n=1)	(Pinto et al., 2018)
Computational study (n=1)	(Damayanti et al., 2017)

n refers to the number of studies.

4.3 In vivo antidiabetic studies of Annona muricata

Various parts of the plant showed potential antidiabetic activities in murine models. Based on the inclusion criteria, a total of 10 *in vivo* studies were evaluated (Table 4.2). These studies indicated that *Annona muricata* can improve the diabetes parameters in the induced diabetic rats through several possible mechanisms of action.

As depicted in Figure 4.3A, most of these studies (n=7; 64%) were conducted using the extracts of leaves, whereas the other 36% of the studies (n=3) used seed extracts and only one study examined the effect of bark extract. Regarding the extraction solvents (Figure 4.3B), aqueous and methanol were the most frequently used solvent in extracting the bioactive components of this plant (n=4; 36%), followed by ethanol (n=2; 18%), and only one study used oil of the seed (Pinto et al., 2018). aqueous and methanol are preferable solvents most probably due to the total flavonoids and phenol contents especially tannins are higher in methanol-water extract of leaves compared to the ethanolic extract (Agu et al., 2017, Gavamukulya et al., 2014). The most common animal model used to study the antidiabetic activity of this plant was streptozotocin (STZ)-induced diabetic in rats (n=7; 64%). Other models used were alloxan-induced diabetic, clozapine-induced diabetic and glucose preloaded in rats (Figure 4.3C). It has been stated by Ivorra (1989), that STZ-induced hyperglycemia in rats is widely used because it considered a good model for the preliminary screening of active agents against diabetes.

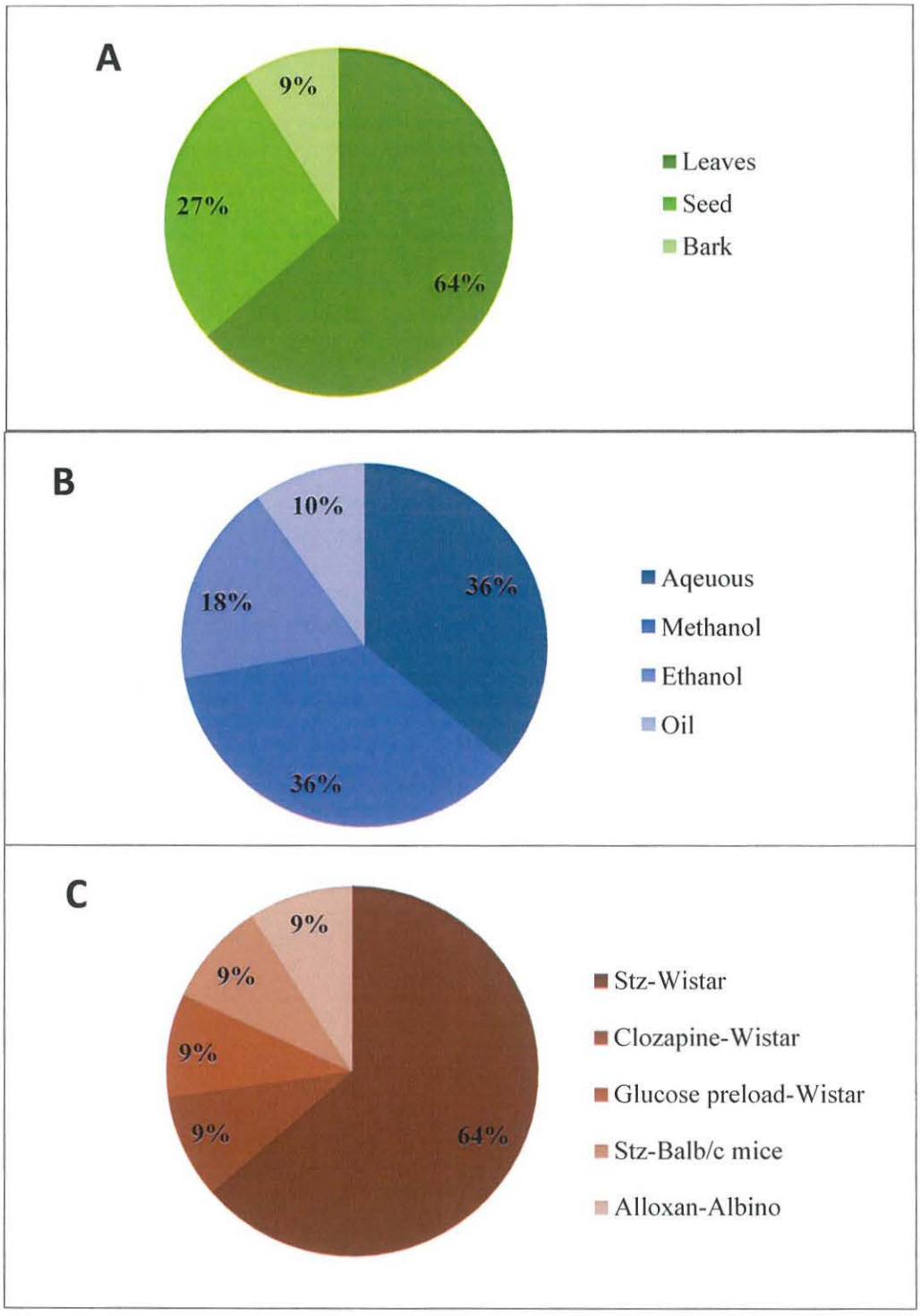

Figure 4.3 Percentage of (A) plant part used, (B) extraction solvent used and (C) animal model used

The leaf extract with a dose of 100 mg/kg was found to maintain the body weight level, significantly decrease the blood glucose levels, improve serum lipid profile by decreasing total cholesterol, triglycerides, low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and increase the level of high density lipoprotein-cholesterol (HDL-C) and percentage of anti-atherogenic index (AAI), restore the altered activity of antioxidant enzymes; reactive oxygen species (ROS), glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and thiobarbituric acid reactive substances (TBARS). also improve the histology and morphology of pancreatic islet by increasing the numerical density of islets (number of islet/pancreas), islet area, islet diameter, numerical density of b-cells (number of β-cells per islet), volume of islets, as well as volume of beta-cells (Adewole & Ojewole, 2009, Adewole & Caxton-Martins, 2006, Adewole, 2010). It is been suggested by (Adeyemi et al., 2010) and (Adewole & Caxton-Martins, 2006) that leaves extract of *Annona muricata* might contain bioactive compounds that exerting anti-hyperglycemic activities through several mechanisms which include stimulating and enhancing the secretion and action of insulin. Furthermore, (Adewole, 2010) reported that leaves of *Annona muricata* have ability to prevent the degeneration of β-cells, regenerate and proliferate the pancreatic β-cells destructed by streptozotocin, suggesting that pancreas contains stable (quiescent) cells; those cells that having capacity of regeneration. Thus, replacing the damage cells and increase the area of insulin immunoreactive β-cells. All the *in vivo* studies on the leaf extract used STZ-induced diabetic rats as a study model.

Apart from the leaf, the seeds have also been reported to possess promising antidiabetic effect by several researchers (Pinto et al., 2018), Found that diabetic rats treated with the