Nanoformulation of *Artemisia afra* and its potential biomedical applications in type 2 diabetes

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A thesis submitted in partial fulfilment of the requirements for the degree Magister Scientiae in the Department of Biotechnology; University of the Western Cape

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December 2019

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KEYWORDS

Type 2 diabetes

Artemisia afra

Nanoparticles

Gold nanoparticles

Green chemistry

Glucose uptake

Traditional medicine

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Abstract

Current research classifies Type 2 diabetes as most prevalent non-communicable diseases in South Africa. Approximately 285 million people are affected globally with an expected increase to 595 million by the year 2035. Synthetic first-line drugs in the treatment of Type 2 diabetes, have been shown to have an efficacy rate of approximately 43% as a result of poor drug uptake and metabolism. Furthermore, given South Africa's uniquely diverse botanical heritage, herbs commonly used traditional medicine have shown promise in the treatment of Type 2 diabetes. *Artemisia afra*, also known as African Wormwood, is one of the most widely used herbs in traditional medicine. This specific species has been extensively studied and is believed to have an effect on glucose uptake in addition to insulin productivity in the body. Drug delivery and uptake systems are important aspects of precision medicine, the science of ensuring medication works optimally for each individual, as they may bypass genetic factors as well as provide optimal efficacy of these treatments. The aim of this study is therefore to produce encapsulated gold nanoparticles from *A. afra*, by phytosynthesis, and determine their effect on glucose uptake in hepatocytes *in vitro* as an alternative and/or additive treatment option. This study has shown that bioactive compounds useful in the treatment of type 2 diabetes may be isolated from *A. afra*. Furthermore, a nano-carrier formulation was produced from these compounds and evaluated in this regard. Stable gold nanoparticle production was observed in this study and it was demonstrated that the nanoparticles produced proved to have a positive effect on glucose uptake *in vitro*. However, further studies are required to determine a toxic dosage of these gold nanoparticles and to successfully isolate and identify the phytochemical responsible for the improved glucose uptake.

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ACKNOWLEDGMENTS

Firstly, I would like to thank and acknowledge our heavenly father for giving me the strength to persevere.

I would also like to thank my supervisor, Dr. Brendon Pearce for giving me the opportunity to do my MSc. project as part of his research group. I am highly appreciative of his guidance, conversations, motivation, advice, patience and freedom to voice my ideas, no matter how odd they may have been, during this project.

Thanks to my co-supervisor, Prof. Mongi Benjeddou for allowing me to use his lab, equipment and allowing me to be part of the precision medicine unit.

To the rest of my colleagues in the Precision medicine unit, I would also like to extend a special word of thanks for always providing a helping hand, word of advice, word of comfort and lifting my spirit with laughter when needed.

I would like to acknowledge and thank my parents, sister and friends for all the love, trust and support they have given me throughout my studies and especially with this project. It has been a long journey but they have always been there to catch me when I fall and it means everything to me.

I would also like to thank the National research fund (NRF), Medical research council (MRC), Chemical Industries Education and training (CHIETA) and the Department of Biotechnology of the University of the Western Cape for providing me with the funding and equipment to complete my studies. I would also like to acknowledge and thank Dr. Leonardo van Zyl for his assistance with the HPLC during my project.

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Lastly, I would like to thank myself for not giving up. This year has been an incredibly tough year and even though it felt like nothing went right, I continued and I pushed through. So, I would like to thank myself, because without my will to succeed, all the motivational words in the world would not have worked.

The research was partially supported by funding from the South African Medical Research Council (SAMRC) through its Division of Research Capacity Development under the Intra-Mural Postdoctoral Fellowship Programme from funding received from the South African National Treasury. The content hereof is the sole responsibility of the authors and do not necessarily represent the official views of the SAMRC or the funders.

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LIST OF ABBREVIATIONS

2DG	2-Deoxy-D-glucos
2DG6P	2-Deoxy-D-glucos Glucose 6-phosphate
ADA	American Diabetic Association
A. afra	*Artemisia afra*
AuNO3	Gold (I) Nitrate
AuNPs	Gold nanoparticles
AuO	Mono-gold Oxide
DM	Diabetes mellitus
DMEM	Basal Medium Eagle Media
EDX	Energy Dispersive X-Ray Analyzer
F1	Fraction 1
F5	Fraction 5

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FBS	Fetal bovine serum
G6P	Glucose 6-phosphate
HEK293	Human embryonic kidney 293
HPLC	High pressure liquid chromatography
IEC	International Expert Committee
IM	Intramuscular
IV	Intravenous
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium
NADH	1,4-Dihydronictinamide adenine dinucleotide
NADP+	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NPs	Nanoparticles
OGTT	Oral glucose tolerance test
PBS	Phosphate Buffered saline
PdI	Polydispersity index
RFU	Relative amount of glucose taken up and metabolized by the cells

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SAMRC	South African Medical Research Council
SC	Subcutaneous
SEM	Scanning electron microscope
T2DM	Type 2 diabetes mellitus
TEM	Transmission electron microscopy
UV	Ultra violet
WHO	World health organisation

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CHAPTER 1

1. Introduction and Literature Review

The prevalence of type 2 diabetes mellitus (DM) has been steadily increasing globally (Abdulfatai, et al. 2012). There is yet to be a cure found for this disease, however lifestyle modifications, management of obesity, oral hypoglycemic agents and insulin sensitizers deliver disease management and may improve a patient's health (Sheen. 2005). Metformin in particular is an insulin sensitizer and recommended as a first line medication, especially in the case of obese patients. It is a biguanide that aids in diabetes treatment by reducing insulin resistance (Collier, et al. 2006).

An estimated 415 million adults were diagnosed and living with DM globally in 2015. This figure is expected to increase to around 642 million people living with diabetes across the world by 2140 (Ogurtsova, et al. 2017).

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1.1. Diabetes

Diabetes mellitus (DM) is a growing medical problem throughout the world that is not only complex in nature but also fast growing in all countries (WHO, 1999). According to the World health organization (WHO) diabetes can be defined as a multifaceted group of disorders that impairs carbohydrates, fat and protein metabolism. This is due to a lack of insulin secretion and/or reduced insulin sensitivity of the tissue (WHO, 1999).

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Secondary pathophysiological changes occur in multiple organ systems due to the metabolic deregulation associated with DM (Dabe and Kefale, 2017). These pathophysiological changes impose an immense burden on diabetic patients, as well as on the health care systems around the world (Anderson, et al. 2015).

There are two main types of diabetes: Type 1 and type 2 diabetes. Type 1 diabetes is caused by an autoimmune destruction of β cells in the pancreas through a T-cell mediated inflammatory response (insulitis) as well as a humoral (B cell) response (Kharroubi and Darwish, 2015). Type 2 diabetes (*T2DM*) is a result of insulin resistance patients and increases insulin-target tissues to demand more insulin (Halban, et al. 2014). An increased demand in insulin coupled with insulin resistance ensures that the increased demand for insulin would not be met by the β cells of the pancreas as these cells are dysfunctional (Kharroubi and Darwish, 2015).

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DM is a persistent global health crisis that has been affecting populations irrespective of their socioeconomic profile or geographic location (WHO, 2016). The rate of morbidity and mortality due to this disorder is annually increasing exponentially, despite many advances in the understanding and management of the metabolic disorder (Dabe and Kefale, 2017).

It is a life-threatening condition and the increase in patients diagnosed with it is due to a number of factors (Patil, et al. 2011). These may include population

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growth, urbanization, aging, mechanized life styles and the increasing prevalence of obesity throughout the globe (Patil, et al. 2011).

1.1.1. Epidemiology of diabetes

Globally, the number of people living with *T2DM* is constantly increasing, with 80% of these people living in low- and middle-income countries (Global burden of diabetes. 2011). However, *T2DM* incidence varies substantially from one geographical region to another due to various environmental and lifestyle risk factors (Abdulfatai, et al. 2012).

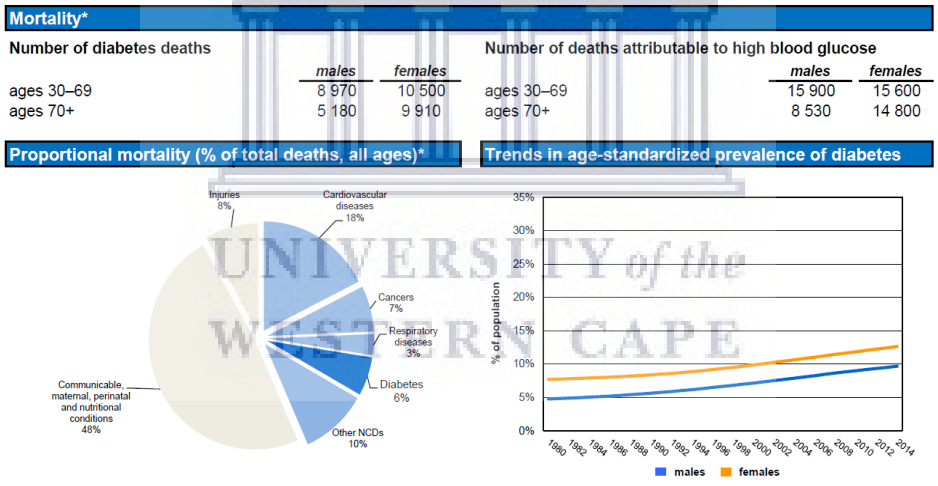

Figure 1.1. Mortality and age-standardized prevalence of diabetes statistics in South Africa (WHO, 2019).

The World Health Organization (WHO) estimates that about 6% of South African deaths are caused by diabetes (**Figure 1**) and this number is expected to

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increase over the next few years. WHO also estimated that Asian countries that are more densely populated, such as India and China, will have an expenditure of more than US$1 trillion annually for the treatment of T2DM and its complications (Dabe and Kefale, 2017). This is due to the huge increase of diabetic cases foreseen by 2030 (Wild, et al. 2004).

1.1.2. Type 2 Diabetes Mellitus

The characterization of *T2DM* by insulin insensitivity due to its insulin resistance leads to a decrease in glucose transport to its target cells, such as the liver, muscle cells, and fat cells, ultimately leading to an increase in fat breakdown through hyperglycemia (Fujioka. 2007). This dysfunction is not suppressed with a meal and leads to a rise of glucagon and hepatic glucose levels (Abdulfatai, et al. 2012).

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1.1.3. Screening and diagnosis of type 2 diabetes

Screening and diagnosis of *T2DM* is based on the American Diabetic Association (ADA) guidelines of 1997 or World Health Organization (WHO) National diabetic group criteria of 2006 (Abdulfatai, et al. 2012). It is based off a single raised glucose reading with symptoms, such as polyuria, polydipsia, polyphagia and weight loss (Expert Committee on the Diabetes and Classification of Diabetes Mellitus, 1997). If this is not the case, it is based on raised values on two occasions (Cox and Elelman, 2009). This may be of either

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fasting plasma glucose (FPG) =7.0 mmol/L (126 mg/dL) or with an oral glucose tolerance test (OGTT), which is done two hours after the oral dose a plasma glucose =11.1 mmol/L (200 mg/dL) (Cox and Elelman, 2009).

The International Expert Committee (IEC) recommended in 2009 that additional diagnostic criteria of an HbA1c result = 6.5% for DM be used. This committee suggested that the term pre-diabetes be phased out completely, however identified the range of HbA1c levels = 6.0% and <6.5% be used to identify individuals at high risk of developing DM (International Expert committee, 2009).

1.2. Disease Management

A number of therapeutic measures are currently in place to treat *T2DM* (Dabe and Kefale, 2017). These include the use of insulin and other agents, such as amylin analogues, alpha glycosidase inhibitors, sulphonylureas, and biguanides (Ghazanfar, et al. 2014). These drugs also often run the risk of having certain adverse effects (Dabe and Kefale, 2017). At higher doses, many of these therapeutics often cause hypoglycemia, liver problems, lactic acidosis and diarrhea (Ghazanfar, et al. 2014). Many herbal medicines have been recommended over many years for the treatment of diabetes by people worldwide (Dabe and Kefale, 2017). Herbal drugs are often more effective and have fewer side effects than other therapeutics. It is also relatively low cost and more easily available (Korkmaz and Gürdal, 2002)

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1.2.1. Biguanides and Metformin

Biguanides are drugs that have multiple functions related to glucose in the body (Abdulfatai, et al. 2012). Its functions include suppressing hepatic glucose production, increasing insulin sensitivity, enhancing glucose uptake by phosphorylating the GLUT-enhancer factor, increasing fatty acid oxidation, as well as decreasing the absorption of glucose from the gastrointestinal tract (Collier, et al. 2006). Metformin, more commonly known as Glucophage, is the most commonly used biguanide and is especially used in overweight and obese patients (Collier, et al. 2006).

Published research from 2008 showed another metformin mechanism of action where AMP-activated protein kinase is activated (Kim, et al. 2008). This enzyme plays a role in hepatic gluconeogenic gene expression (Abdulfatai, et al. 2012). Metformin should however be used with great caution in elderly diabetic patients with signs of renal impairment, as the drug may cause the development of lactic acidosis (Collier, et al. 2006).

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1.2.2. Insulin

Insulin may either be used as monotherapy or in combination with oral hypoglycemic agents (Chiniwala and Jabbour. 2011). Augmentation therapy with basal insulin is often very useful in the case of some remaining pancreatic beta cell function (Mayfield and White. 2004).

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Insulin therapy is however limited with regards to mimicking normal physiologic insulin secretion (Zhu and Zhang, 2016). Traditional intermediate- and long acting insulins such as NPH insulin, lente insulin, and ultralente insulin may not always be a perfect option for treatment (Zhu and Zhang, 2016). This is due to them being inconsistent in absorption and peaks of action that may often result in hypoglycemia (Zhu and Zhang, 2016).

1.3. Herbal medicine

Herbal medicines and phytonutrient or nutraceutical usage have been rapidly expanding across the world (Ekor. 2014). Many people have resorted to herbal products for the treatment of various health problems in different national healthcare settings (WHO. 2004). Over recent years a surge in acceptance and public interest in natural therapies have occurred in both developing and developed countries (Bodeker, et al. 2005). Herbal use is viewed as an integral part in many cultures and is often the primary source of healthcare in traditional medicine (Ekor. 2014). For this reason, it is estimated that about 80% of the global population, especially those in developing countries, make use of herbal medicine (Bodeker, et al. 2005).

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1.3.1. Herbal/Traditional medicine in SA

South Africa is considered a botanical “hotspot” as it is rich, unique and diverse botanical heritage (Liu, 2009). Though South Africa is a small country and its land surface makes up less than 1% of the Earth (Coetzee et al. 1999), however statistical studies have shown that approximately 25% of the total number of

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higher plants in the world may be found in South Africa (Van Wyk, et al. 2000). It is estimated that South Africa is home to about 3000 plants used in traditional medicine by traditional healers (Coetzee et al. 1999). An industry that boasts about 27 million consumers (Liu, 2009). Only about 350 species are most commonly used in traditional medicine and about 38 of these indigenous species have been commercialized to an extent (Van Wyk, et al. 2000).

1.3.2. DM treatment using traditional medicine

DM management with medicinal plants in combination with dietary restrictions has been a field of interest for many researchers (Li, et al. 2015). In fact, natural sources form a basis for most modern drugs (Dabe and Kefale, 2017). At least one active ingredient found in plants, can be found in about 25% of all prescriptions (Li et al. 2015). There is a strong need for alternative strategies to complement those of current modern pharmacotherapies of DM (Tahraoui et al. 2007). While herbal drugs are known to be a significant factor in conventional medicine, literature has shown that more than 400 species of plants exhibit some sort of anti-diabetic activity (Tahraoui et al. 2007).

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1.4. *Artemisia afra*

According to WHO, approximately 80% population of the population in Asian and African countries are dependent on traditional medicine for treatment of various infectious and chronic conditions (Patil, et al. 2011). This system medicine is popular due to people's faith in traditional methods that has been

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passed down for generations, its accessibility and affordability (Patil, et al. 2011).

There are many different common or local names describing this plant (Liu, et al. 2009). This is due to the widespread of the use of the plant and also the large number of different ethnic groups within South Africa (Watt and Breyer-Brandwijk, 1932). In the Xhosa language it is called "Umhlonyane", in the Zulu language "Mhlonyane", in Sotho it is referred to as "Lanyana", in it is called Tswana "Lengana", in English "African wormwood" and in Afrikaans "Wilde als" (Liu, et al. 2009).

Common reasons for ingesting or taking *A. afra* as treatment are respiratory ailments and stomach pain (Van Wyk, 2011). It is a common medicinal herb plant to be cultivated in home gardens around South Africa and rooted cuttings may be available for purchase at most retail nurseries (Graven, et al. 1990). The essential oil of *A. afra* often varies in constituents, however always contains 1,8-cineole (eucalyptol), a-thujone, ß- thujone, camphor and borneol as the main constituents, along with chrysanthenyl acetate and other sesquiterpenoids (Graven, et al. 1990).

Though *A. afra* is an extremely popular and very commonly used herbal medicine in Southern Africa, research conducted on this species has been limited (Van Wyk, 2008).

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1.4.1. Botanical classification and morphology

Artemisia afra is a tall plant that can grow up to a height of 2 meters tall and may be described as a perennial woody shrub with a leafy, hairy and ridged stem (Van Wyk, et al. 1997). The leaves are dark green on the adaxial surface, while lighter green on the abaxial surface. The leaves reach 8 cm in length, 4 cm in width and are soft in texture (Liu, 2009).

The *A. afra* plant produces yellow, butter-coloured flowers with many bracts and its normal blossoming period is between January and June (Hilliard, 1977). The plant has an aromatic and pungent odour that is easily identifiable and sweet after bruising. The fruit bared by the plant is approximately 1 mm in length and has a silvery-white coating. The plant's shape is curved and slightly 3-angled (Hilliard, 1977; Van Wyk, et al. 1997).

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1.4.2. Geographical distribution

A. afra grows in the high land areas of Eastern and Southern Africa (Patil, et al. 2011). The altitudes range between 1500 and 3000m where the soils have a vast range from volcanic ash, loamy sands, to sandy or calcareous clay loams of volcanic or granitic origin (Patil, et al. 2011).

The geographical location of the *A. afra* have appeared to affect the constituents which appear as the major components in the oil of the *A. afra* (Braünlich et al.

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2018). As an example, in a study conducted by Oyedeji et al., plant material was collected from three different provinces of South Africa (Braünlich et al. 2018). It was found that each of the plant populations were dominated by a different constituent (Oyedeji. 2009). Namely 1,8-cineole, a- or ß- thujone, or camphor (Braünlich et al. 2018).

1.4.3. Chemical variation in *A. afra*

The volatile secondary metabolite content and concentration vary frequently within *A. afra* and is caused by a number of factors which include the geographical area the plant originated from, the different parts of the plant used, drying methods and variation within the plant population (Liu, et al. 2009).

Oils extracted from different parts of the plant have also shown to vary in chemical components (Dube, 2006). Constituents such as camphor, a wax ester, triacontane, scopoletin and quebrachitol were found in the oil of the flowering parts of the plant (Liu, et al. 2009). The oils extracted from the roots have shown to contain isomeric coumarins and five acetylenes (Liu, et al. 2009). Also, the aerial parts of the plant contained oil that had thujone and umbelliferone-derivatives (Mwangi et al., 1995).

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Cytotoxicity studies of ethanol extracts of *A. afra*, followed by bio-assay guided fractionation has led to the isolation of a sesquiterpene lactone, also known as isoalantolactone (Spies, et al. 2013). This is considered the main bioactive compound in the plant with regards to its anti-diabetic effects (Braünlich et al. 2018).

Figure 1.2. A.afra plant branch (A) and fully grown plant (B) (Coetzee et al. 1999).

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1.4.4. Pharmaceutical dosage form

Artemisia afra is not only used in South African traditional medicine, also widely used in many other parts of the world as well (Eshetu, et al. 2016). Traditional healers use the plant extracts either alone or in combination with other medicinal plants to treat a variety of ailments ranging from simple headaches to neurological disorders (Mander. 1998). In South Africa, the plant is employed

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as a remedy for a number of ailments, which include chest conditions, coughs, colds, heart burns, hemorrhoids, fevers, malaria, asthma, and other conditions (Cunningham, et al. 1996). However, in different countries, the plant is also used for many other ailments, depending of the type of extraction method and compounds extracted (Eshetu, et al. 2016).

It is ingested in a number of ways and which makes use of various preparation methods (Issa and Bule, 2015). These include infusions, decoctions, molasses, and alcohol extracts of the plant (Burits, 2001). These extractions are then used in the treatment of coughs, chills, colds, stomach ache, dry dyspepsia, diabetes, as a purgative and even a cure for smallpox and Malaria (Issa and Bule, 2015).

In the past, researchers attempted to formulate and evaluate tablets produced using the *A. afra* plant (Patil, et al. 2011). It was concluded that dried aqueous extract of *A. afra* leaves are unfortunately problematic to work with and very hygroscopic (Komperlla. 2004). However, it was possible to manufacture and produce pharmaceutical quality tablets under controlled humidity conditions (Patil, et al. 2011).

The most common way, however, to ingest the plant is to inhale the steam produced from boiling the *A. afra* leaves in water or as a tea infusion (Van Wyk. 2002). Another common way of utilizing the *A. afra* leaves is to put some leaves in socks (Von Koenen. 2001). The leaves have been known to prevent sweating (Braünlich et al. 2018).

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As an infusion, or tea a quarter cup of fresh leaves is generally added to a cup of boiling water containing a quarter cup of fresh, or dried, leaves is allowed to draw for about 10 minutes (Liu, 2009). The mixture is strained and sweetened with honey before taken orally (Roberts, 1990).

Studies on drying methods have revealed that there is no significant loss of volatile constituents that takes place during air-drying, oven-drying or sun-drying, however there is a marginal loss of sesquiterpenoids during sun-drying (Braünlich et al. 2018).

1.5. Drug delivery methods

The drug bioavailability of a medication is directly affected by the route of administration (Ruiz and Sciolo Montoto, 2018). This drug bioavailability determines the start and duration of the pharmacological effect of the medication (Ruiz and Sciolo Montoto, 2018). A number of factors need to be taken into consideration when designing a dosage form of a drug (Ruiz and Sciolo Montoto, 2018). These include the intended route of administration, the amount or dose intended to be administered, the absorption site and its anatomical and physiological characteristics, the site physiochemical properties and the potential effects of the medication at the site of administration (Ruiz and Sciolo Montoto, 2018).

There are many forms of drug administration, one being the topical route which involves creams, gels and lotions (Verma, et al. 2010), however there are two main

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forms of drug administration, enteral and parenteral drug administration, with parenteral containing various forms of administration (Øie and Benet, 2002).

The enteral route is usually the oral route of drug administration (Ruiz and Sciolo Montoto, 2018). However, the enteral routes also include the rectal, vaginal and urethral routes (Verma, et al. 2010). It is a convenient and economical way of administering drugs and is often the first choice for drug administration (Ruiz and Sciolo Montoto, 2018). Orally administered drugs move through the intestinal and digestive tract in the same way food would (Verma, et al. 2010). Unabsorbed compounds from the metabolized drug leave the body through the anal sphincter and the total transit time within the body can range from 6 hours to 5 days (Ruiz and Sciolo Montoto, 2018).

Parenteral drug administration is carried out with the drug administered directly through the skin, in or towards systemic circulation (Verma, et al. 2010). There are three forms of parenteral routes; intravenous (IV), intramuscular (IM) and subcutaneous (SC) (Verma, et al. 2010). The IV route involves a drug solution being passed through a needle, directly into a vein (Øie and Benet, 2002). The IM route also makes use of a needle; however, the drug is injected into muscle tissue (Boylan and Nail. 2002). The SC route consists of injecting the drug under the skin and directly into the adipose layer beneath the dermis of the skin (Mishra and Stringer. 2010). In cases where drugs cannot be efficiently absorbed orally or becomes unstable once reaching the intestinal tract, parenteral drug administration is preferred (Ruiz and Sciolo Montoto, 2018).

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1.5.1. Drug delivery limitations

As with most medical advances, there are drawbacks to commonly used drug administration routes (Ruiz and Sciolo Montoto, 2018). The main drawback for the parenteral route being that it is irreversible and may initiate fear and pain in patients (Øie and Benet, 2002). It may also lead to tissue damage or infections (Verma, et al. 2010).

Special care must always be taken when selecting a suitable vehicle used to solubilize a drug in IV administrations (Lim et al., 2014). Poorly solubilized drug precipitation may occur during injections and may prevent the drug from mixing into circulation (Lim et al., 2014). It is also very important to always perform injections slowly (Ruiz and Sciolo Montoto, 2018).

Though IM administration of drugs are normally safer than that of IV, IM application errors during injections may lead to blood clots, scars, abscesses and nerve damage (Mishra and Stringer. 2010). Precipitation at the injection site is also a risk and it is important to solubilize the drug fully, as in I administrations (Fontes Ribeiro. 2005).

Oral route absorption of drugs is highly dependent on the physiological state of the gastrointestinal tract (Ruiz and Sciolo Montoto, 2018). This in turn is affected by factors such as diet, hormones, autonomic nervous system, pathological states and other drugs that may interact (Ruiz and Sciolo Montoto, 2018).

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1.5.2. Drug delivery systems

Colloidal drug carrier systems are a promising area in drug delivery systems (Reddy and Swarnalatha, 2010). Colloidal drug carrier systems include micellar solutions, vesicle and liquid crystal dispersions and nanoparticle dispersions that consist of small particles usually between 10-400nm in diameter (Reddy and Swarnalatha, 2010). The goal of developing these drug delivery systems is to optimize drug loading and releasing properties, while maintaining a stable and long shelf-life together with low toxicity (Reddy and Swarnalatha, 2010).

Great success has already been found through applying nanoparticles and nano formulations as drug delivery systems (Reddy and Swarnalatha, 2010). It has been used in anti-tumour therapy, gene therapy, AIDS therapy, radiotherapy, antibiotics, virostatics, protein delivery and vaccines and as vesicles able to pass the blood-brain barrier (Reddy and Swarnalatha, 2010). Nanoparticles provide numerous advantages with regards to drug targeting, delivery and release (Reddy and Swarnalatha, 2010). They also provide the additional potential to combine diagnosis and therapy, allowing them to advance as a major tool in nanomedicine (Reddy and Swarnalatha, 2010).

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1.6. Nanoparticles

Nanotechnology platforms can help overcome associated challenges with the delivery of many active pharmaceutical or herbal ingredients, such as increase the uptake of these ingredients, and broaden study mechanisms (Sharma et al. 2017).

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Nanoparticles (NPs) are materials of various types produced at a nanoscale level (Khan, et al. 2017). These NPs include particulate substances with at least one dimension that is less than 100nm (Khan, et al. 2017). Among all nanoparticles, gold nanoparticles (Au NPs) have been of interest due to their unique surface morphologies, controlled geometry and stable nature (Nadeem, et al. 2017). Au NPs possess unique properties and multiple surface functionalities; therefore, their biocompatible nature allows them to be suitable for medical applications such as cancer, arthritis and various antimicrobial therapies (Yeh, et al. 2012). AuNPs produced through green synthesis have been used to develop biosensors, disease markers and the quantification of blood glucose (Nadeem, et al. 2017). However tedious purification steps, greater sizes of nanoparticles and a poor understanding of the underlying mechanisms are some of the major drawbacks associated with green synthesis, therefore it is critical that fast and clean synthesis of nanoparticles is optimized to produce nanoparticles displaying desired sizes and morphologies (Shukla and Iravani, 2017). Naturally biodegradable materials, as a source of nanoparticles through green synthesis, is a highly promising area in nanotechnology (Shukla and Iravani, 2017). Materials may include microorganisms, enzymes, vitamins, polysaccharides, biodegradable polymers and plant extracts (Shukla and Iravani, 2017). Modern developments in science and industry have implemented green synthesis as part of the global efforts to reduce hazardous waste production (Sharma, et al. 2009). Through green synthesis of the nanoparticles, a simple process of mixing metal salt with the plant is required. The process then completes itself at room temperature in minutes to hours (Nadeem, et al. 2017).

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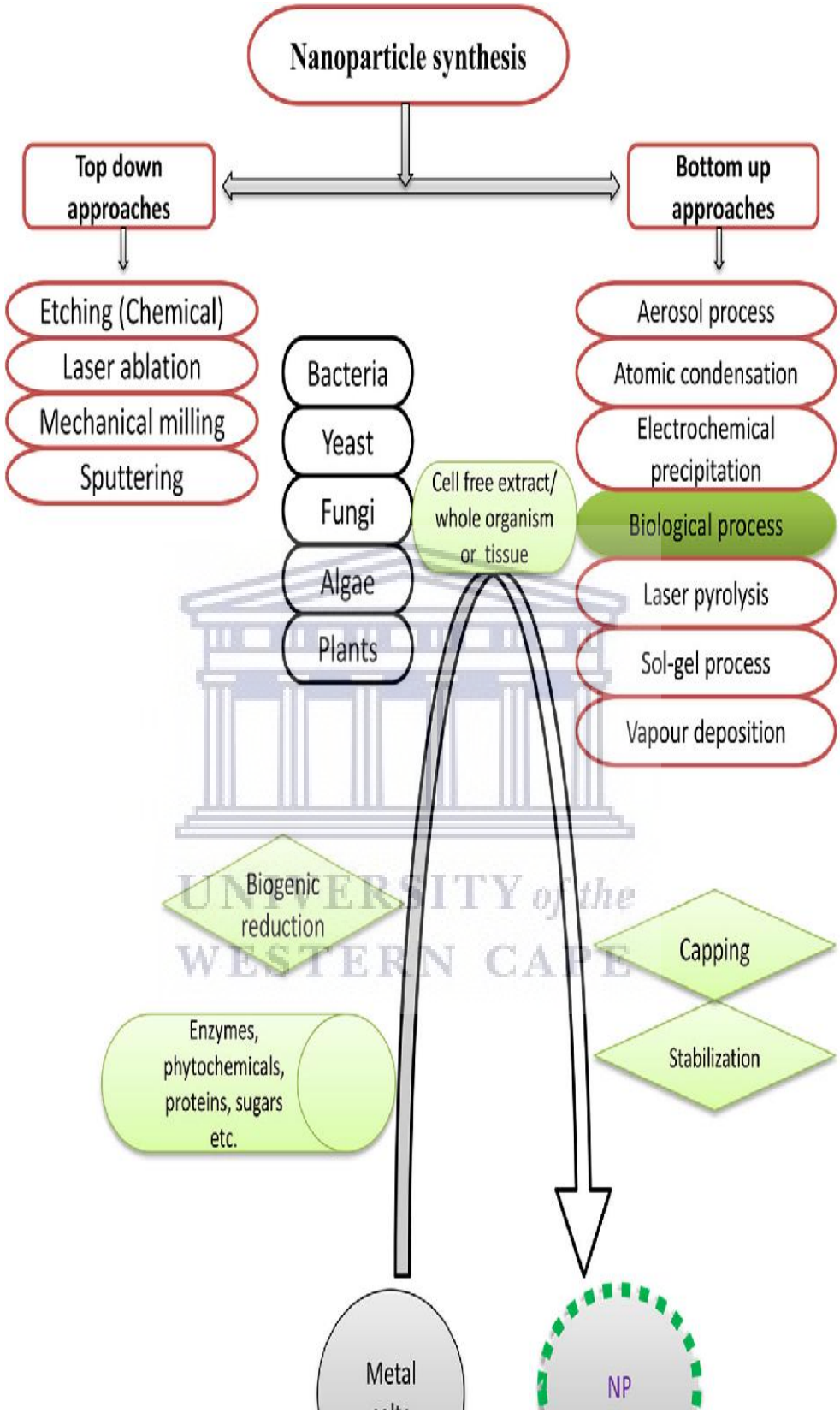

Figure 1.3. Various methods of nanoparticle synthesis (Hussain et al. 2016).

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Aqueous extract from a range of medicinal plant extracts have been shown to contain different bioactive compounds (Khaled et al. 2017). These bioactive compounds act as non-toxic capping, as well as reducing agents for green synthesis of metal and metal oxide nanoparticles (Khaled et al. 2017). Noble metal nanoparticles possess a range of biological applications which include in vitro antioxidant potential, antimicrobial power and cytotoxicity towards different cancer cells (Francis et al. 2017).

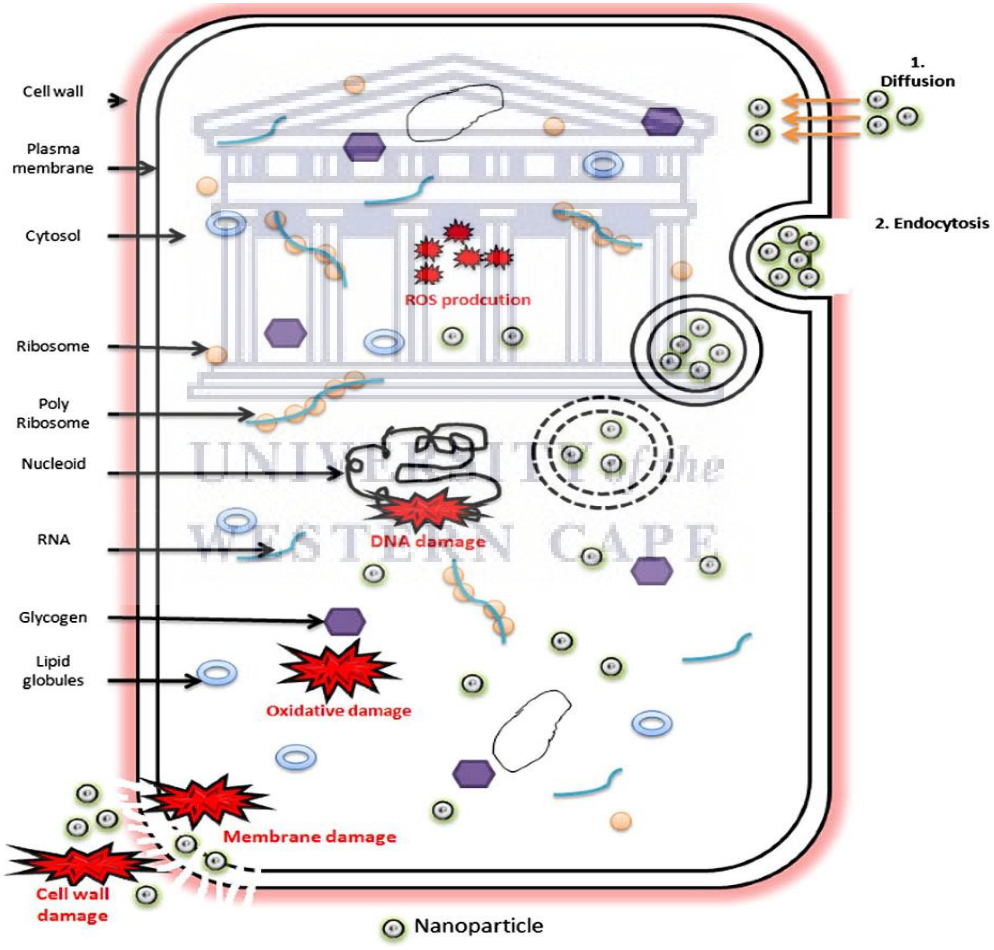

Figure 1.4. Methods of nanoparticle uptake by cells (Hussain et al. 2016).

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Phenolic acid, flavonoids, terpenoids and alkaloids are of the many novel secondary metabolites found in plant crude extract that are responsible for the reduction of ionic into bulk metallic nanoparticles formation (Aromal, et al. 2012). Eco-friendly nanosized particles are synthesized through the redox reactions that take place involving these primary and secondary metabolites (Kuppusamy, et al. 2016). This bioreduction in the presence of metabolites and redox enzymes takes place through Au+ ions being reduced into metallic Auo nanoparticles (Kuppusamy, et al. 2016).

1.7. Aim

The aim of this study is to produce encapsulated gold nanoparticles from *A. afra*, by green synthesis, and determine their effect on glucose uptake in hepatocytes *in vitro* as an alternative and/or additive to Metformin in patients with reduced treatment response.

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CHAPTER 2

2. Materials and Methods

2.1. Plant extracts preparation

A. afra plants were grown and harvested at full maturity in the month of March 2018 and healthy leaves were collected. The leaves were rinsed using running tap water and shade-dried at room temperature (28 ± 2 ◦C) on tissue paper. The air-dried leaves were grounded to a fine powder by a mortar and pestle. Roughly 100 grams of dried powder was added to 1L of distilled water in a bottle. The mixture was stirred in a shaking incubator at 75° C for 1 hour. The extract was recovered through Whatmann filter paper (Lasec, Ndabeni, Cape Town; South Africa).

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A volume of 50ml of pure extract was freeze dried (Labconco Corporation, Kansas City, Missouri; United States) to remove impurities and the resulting powder was resuspended in 50ml distilled water. Freeze drying also known as lyophilization, is the process of water removal from a frozen product through the use of a high-pressured vacuum (Bhambere, et al. 2015). This is done by converting the ice in the product directly from solid phase to vapor without going through the liquid phase (Bhambere, et al. 2015).

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2.2. High Pressure Liquid Chromatography (HPLC)

A volume of 200µl of this pure extract was fractionated by HPLC, resulting in 8 fractions, while the rest of the pure extract was refrigerated at 4°C for further use. High pressure liquid chromatography, also referred to high performance liquid chromatography, is a specific form of column chromatography commonly used to separate, quantify or identify active compounds in a sample/solution (Maviya, et al. 2010). The HPLC makes use of a column holding packing material (stationary phase), a pump that moves the solvent (mobile phase) through the column and a detector used to detect and indicate the retention times of the various molecules (Maviya, et al. 2010).

2.3. Biosynthesis of gold nanoparticles

It is generally accepted that the ability of plant extracts to reduce gold salt to gold nanoparticles is dependent on the presence of easily oxidized phytochemicals (Oueslati et al. 2018). Moreover, these phytochemicals often have the ability to play dual roles during gold nanoparticle synthesis, acting as both reducing and capping agents (Choi et al. 2014; Santhoshkumar et al. 2017; Biao et al. 2018; Oueslati et al. 2018).

The gold nanoparticles were synthesized using 0.1 M gold nitrate solution (Sigma-Aldrich, St. Louis, Missouri; United States) and extract of *A. afra*. Both the solutions were amalgamated to initiate the reduction of AuNO3 solution into Au0. The mixture was agitated constantly on a magnetic stirrer. Color changes were

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detected from dark yellow to red-brown by the naked eye. The reaction was stopped by the removal of the mixture from the magnetic stirrer and bath sonicated immediately for 1 hour. The obtained *A. afra* gold nanoparticles were filtered using a 0.22 filter and stored in 2ml Eppendorf tubes (Lasec, Ndabeni, Cape Town; South Africa) at room temperature for further characterization.

2.4. Size Optimization of AuNPs

Various methods were used in order to optimize the production of gold nanoparticles. Size optimization parameters included temperature at which the nanoparticles were synthesized, time length of synthesis, and amount of light the solution was exposed to while synthesis took place. After optimization the most successful nanoparticles produced with the desired properties (smaller than 150nm) were synthesized at room temperature (28 ± 2 ◦C) on benchtop while stirring.

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2.5. Characterization of AuNPs

2.5.1. UV–vis spectroscopy (zeta-sizer)

All the absorbance-based experiments and formation of AuNPs were carried out using a UV–vis spectrophotometer (UV- 7504) (Malvern Panalytical, Malvern; United Kingdom). For this purpose, 300 mL of gold nanoparticles were taken in a quartz cell (Sigma-Aldrich, St. Louis, Missouri; United States) of 1.0 cm path length and the volume brought up to 2 mL by the addition of deionized water. The

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absorbance of gold nanoparticles was recorded in the wavelength ranging from 300 to 700 nm at different time intervals (Rasheed, et al. 2017).

2.5.2. SEM and EDX analysis

The surface morphologies of the AuNPs were studied using a field emission scanning electron microscope (Siemens, Munich; Germany), with an accelerating voltage of 1 kV or 5 kV. The samples for SEM observations were prepared by mounting small amount of gold nanoparticles onto the surfaces of clean silicon chips (Sigma-Aldrich, St. Louis, Missouri; United States) followed by coating with a thin film of gold using a gold sputtering device before measuring. The operating pressure of 7× 10−2 bar and deposition current of 20mA for 2 min was applied for current analyses. Elemental analyses of the gold nanoparticles were performed using an energy dispersive X-ray (EDX) detector (Siemens, Munich; Germany) (Rasheed, et al. 2017).

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2.5.3. TEM analysis

The stable gold nanoparticles were thoroughly washed and diluted with deionized water to achieve the absorbance in the range of 0.50 a.u. One drop of diluted nanoparticles was placed onto a carbon-coated copper grid (Sigma-Aldrich, St. Louis, Missouri; United States) and vacuum-dried, before AuNPs were envisaged using a high-resolution transmission electron microscope (Siemens, Munich; Germany) at a voltage of 120 kV (Rasheed, et al. 2017).

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2.5.4. MTT assay for cytotoxicity analysis

The cytotoxic effect of gold nanoparticles on human embryonic kidney (HEK293) cells (Sigma-Aldrich, St. Louis, Missouri; United States) was assessed following the MTT protocol where cells were cultivated in DMEM (Sigma-Aldrich, St. Louis, Missouri; United States) with additionally 10% FBS (Sigma-Aldrich, St. Louis, Missouri; United States) at 37◦C in 96-well microtiter plates (Sigma-Aldrich, St. Louis, Missouri; United States). The plates were exposed to varying doses of gold nanoparticles (20–140 g/mL) for 24 h, 48 h and 72 h. Cells without nanoparticles served as negative control. After AuNPs treatment, the medium was separated, and cells were incubated with 200µl of MTT (Sigma-Aldrich, St. Louis, Missouri; United States) in fresh medium at 37◦C for 4h. The resultant formazan crystals from the mitochondrial reduction of MTT (Sigma-Aldrich, St. Louis, Missouri; United States) were solubilized in DMSO (Sigma-Aldrich, St. Louis, Missouri; United States). The absorbance of each sample was determined using a microplate absorbance reader (Promega, Madison, Wisconsin, United States) at 570 nm and percentage of cell viability was calculated using the following equation:

Cell viability (%) = (Absorbance of test/Absorbance of control) × 100

2.6. Glucose uptake assay

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The protocol used for measuring glucose uptake in mammalian cells is based on the detection of the glucose analog, 2-deoxyglucose-6-phosphate, in the form of a kit produced by Promega (Promega, Madison, Wisconsin, United States).

A volume of 10ų 2DG (Promega, Madison, Wisconsin; United States) was diluted in 990ųl PBS (Sigma-Aldrich, St. Louis, Missouri; United States). The reaction mixture was produced according to manufacturer instructions as follows:

Reagent	1 Reaction (ųl)	50 Reactions (ųl)
Luciferase reagent	100	5000
NADP+	1	50
G6PDH	2.5	125
Reductase	0.5	25
Reductase substrate	0.0625	3

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The reaction mixtures were incubated at room temperature for 1 hour. After 1 hour the cells were incubated with various concentrations of nanoparticles, the cells were washed with 100ųl PBS (Sigma-Aldrich, St. Louis, Missouri; United States). A volume of 50ųl 1mM 2DG (Promega, Madison, Wisconsin; United States) was added to each well and allowed to incubate for 10 minutes. A volume of 25ųl stop buffer (Promega, Madison, Wisconsin; United States) was added to each well and shaken briefly. Thereafter, 25ųl of neutralization buffer (Promega, Madison, Wisconsin; United States) was added to each well and the wells were shaken briefly. and shake briefly. Finally, a volume of 100ųl of mix made in step

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2 was added and the plate shaken briefly. The plate was then incubated at room temperature for 30 minutes and read on a plate reader (Promega, Madison, Wisconsin, United States) at 15-minute intervals for 2 hours.

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CHAPTER 3

3. Results and Discussion

3.1. Plant Extract Preparation

The main principle of freeze drying is the removal of water and is made possible by the phenomenon known as sublimination (Bhambere, et al. 2015). Sublimination of water occurs at pressures and temperatures below triple point (Bhambere, et al. 2015). Samples are frozen prior to freeze drying and then exposed to a high vacuum in the presence of heat (Bhambere, et al. 2015). This results in frozen liquid being sublimated and only solid, dried components of the original liquid or sample remains (Bhambere, et al. 2015). The driving force for the removal of water during lyophilization is the concentration gradient of the water vapor that forms between the drying front and the condenser (Bhambere, et al. 2015). The pressure of the vapor rises as the temperature rises during primary drying (Bhambere, et al. 2015). For this reason, the primary drying temperature should remain as high as possible, though below the critical process temperature, in order to avoid possible loss of cake structure (Bhambere, et al. 2015). This is the critical process temperature and is the collapse temperature for amorphous substance, or eutectic melt for the crystalline substance (Bhambere, et al. 2015).

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Figure 3.1. Rehydrated freeze dried *A. afra* extract using distilled water.

Ice crystals start separating out, during freeze drying, until the solution becomes concentrated to its maximum (Bhambere, et al. 2015). Upon further cooling of the sample, phase separation of the ice and solute takes place (Bhambere, et al. 2015).

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3.2. High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a system of column chromatography that pumps a sample mixture or analyte in a solvent (mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). The retention time is dependent on the interactions between the molecules being analysed, the stationary phase and the solvents used

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(Maviya, et al. 2010). The sample of interest is introduced to the HPLC system in a small volume to the stream of the mobile phase (Maviya, et al. 2010). This stream is then retarded by specific interactions with the stationary phase that may be chemical or physical interactions (Maviya, et al. 2010). The rate and amount of retardation is dependent on the nature of the analyte and composition of the mobile and stationary phases (Maviya, et al. 2010). The retention time is referred to the time it takes for a specific analyte to elute or exit the column (Maviya, et al. 2010). Solvents commonly used in HPLC include any miscible combinations of distilled water or organic liquids (Maviya, et al. 2010). Gradient elution is when separation is done in order to vary the mobile phase composition during analysis (Maviya, et al. 2010).

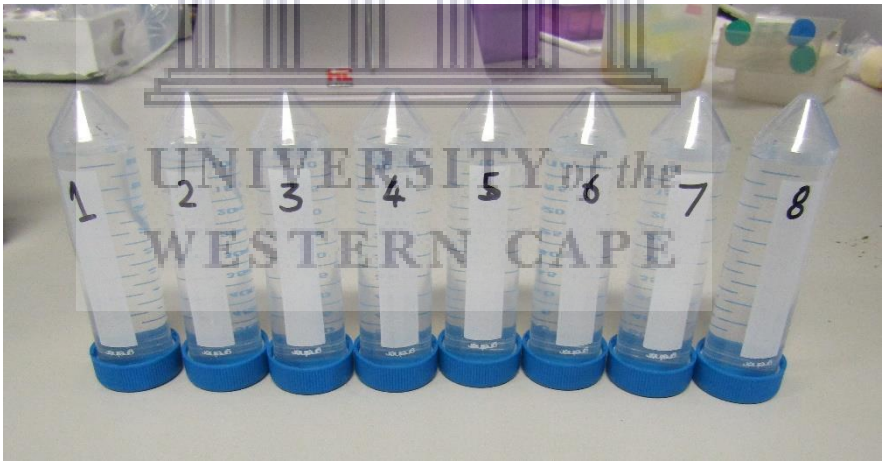

Figure 3.2. *A. afra* fractions produced by the HPLC.

Often a mobile phase of changing or different compositions is used (Bird, 1989). This is to ensure that in the beginning of the HPLC the method is not as effective in dissolving the molecules of interest as the solid phase would be (Bird, 1989). Consequently, the molecules remain in the solid/stationary phase for most of

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their time (Bird, 1989). As the liquid phase composition changes, it becomes more and more efficient in dissolving the molecules of interest and they are increasingly removed from the stationary phase and travels with the mobile phase (Bird, 1989). This is called the gradient elution (Bird, 1989). Different molecules or sets of molecules are then captured and hence the sample of interest is fractionated (Bird, 1989). The fractions generated by this process were each used to synthesized AuNPs, by green synthesis. Each set of particles was then referred to by their fraction number for the remainder of the study.

3.3. Characterization of nanoparticles

Biocompatibility is an essential property in the design of drug delivery systems. A general definition of a biocompatible surface is that it should not prompt undesired responses from the organism. Biocompatibility is alternatively defined as "the ability of a material to perform with an appropriate response in a specific application" (Williams, 2003; Keck and Müller, 2013). It is, therefore, imperative to identify the properties, to understand the mechanisms by which nanomaterials interact with living systems and thus to understand exposure, hazards and their possible risks. The pharmacokinetics and distribution of nanoparticles in the body depends, largely, on three key factors; surface physicochemical characteristics, shape and size. For instance, nanoparticles with 10 nm in size were preferentially found in blood, liver, spleen, kidney, testis, thymus, heart, lung, and brain, while larger particles (>100nm) are detected only in spleen, liver, and blood (De Jong et al., 2008; Adabi et al., 2017).

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Similarly, the surface of nanoparticles also influences their distribution in these organs, as they combine with serum proteins available in systemic circulation, thus influencing their cellular uptake. It should, at this point, be emphasized that a biocompatible material generates no immune response. An assessment of the *in vivo* protein profile is therefore crucial to address these interactions and to establish biocompatibility (Keck et al., 2013). Lastly, the clearance of nanoparticles is also size and surface dependent. Small nanoparticles (<20-30 nm) are rapidly cleared by renal excretion, while 200 nm or larger particles are more efficiently taken up by mononuclear phagocytic system (reticuloendothelial system) located in the liver, spleen, and bone marrow (Moghimi et al., 2001; Adabi et al., 2017).

The Polydispersion Index (PdI) is a unit used to estimate the average uniformity of a particle solution (Clayton, 2016). Larger or higher PdI values correspond to a larger size distribution within the particle sample (Clayton, 2016). PdI is also an indication of nanoparticle aggregation throughout the particle sample along with the consistency and efficiency of particle surface modifications (Clayton, 2016). A sample with a PdI value of less than 0.1 is considered monodisperse, where a PdI value of above 0.1 is considered polydispersed (Clayton, 2016).

Table 3.1: Size and poly dispersion index (PDI) of each nanoparticle batch produced.

Fraction	Size (d.nm)	PDI
1	29.39	0.925
2	28.75	0.645

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3	43.93	0.594
4	47.04	0.195
5	12.15	0.915
6	11.20	0.925
7	12.12	0.951
8	54.09	0.773
***A. afra* extract np**	144.0	0.162

The PdI of all nanoparticles produced from the *A. afra* whole extract, as well as each of the eight fractions, were above 0.1. (**Table 3.1**). Therefore, all the nanoparticles produced in this project may be considered polydispersed. The nanoparticles also produced an observed constant voltage of -18mV and as well as a pink hue. It should be recalled that size is an important factor of a drug delivery systems and influences the pharmacokinetics, tissue distribution and clearance of the drug delivery system (Danaei, 2018). Hepatic uptake and accumulation, tissue diffusion, tissue extravasation and kidney excretion all significantly depend on the particle size of the drug delivery system and nanocarriers larger than 150nm in size are unable to enter or exit fenestrated capillaries in the tumour microenvironment and liver endothelium (Danaei, 2018).

Nanoparticles carrying substances, also known as nanocarriers, that circulate in normal blood vessels do not easily leave the capillaries that run through tissues such as the kidneys, lungs or heart, with a diameter ranging from 100–150 nm (Danaei, 2018). The only nanoparticle produced in this project that had a diameter of larger than 100nm were those produced using the crude whole

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extract of the *A. afra*. The tissue of the glomerulus in the kidneys and islets of the pancreas have miniscule pores with diameters ranging between 10–15 nm (Kraft, 2014). Particles with diameters less than 10 nm may be subjected to renal filtration via the wall of the glomerular capillary and are prevented from being reabsorbed (Danaei, 2018). Tissue and capillary pore sizes range between the tissues in the body (Danaei, 2018). For this reason, nanocarriers of 50–200 nm in size remain in their intact form and are unable to escape from continuous blood capillaries (Choi, 2011).

Nanoparticles synthesized through green synthesis by the use of plants and plant extracts are well known to be generally polydispersed with various morphologies and a wide range of size distributions (Nozuri, 2014). However, size, shape and purification of green nanoparticles has been a great challenge for scientists in recent years (Nozuri, 2014). This is revealed in the nanoparticles produced. All the nanoparticles were produced under the same conditions; however, they vary in size. This may have been due to more bioactive compounds being present in

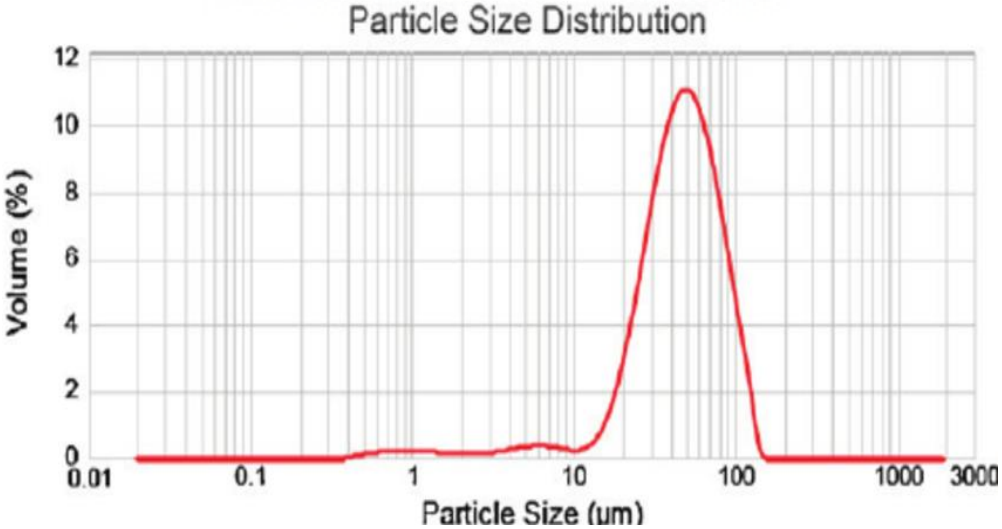

Figure 3.3. Graph showing particle size distribution of a nanoparticle.

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some fractions than in others. It may also have been due to some compounds being reduced and acting as capping agents better than other compounds.

3.3.1. UV-vis Spectra Analysis

UV-visible spectra is a common method to confirm the synthesis of gold nanoparticles (Lee et al. 2019). Gold nanoparticle capping normally induces either a bathochromic (red) or a hypsochromic (blue) shift (Lee et al. 2019).

As the nanoparticles produced in this study had a pink hue a bathochromic shift took place, characteristic of gold nanoparticles (Lee et al. 2019). Absorbance was measured at a number of wavelengths ranging between 450nm and 600nm (**Figure 3.4**). All nanoparticles synthesized from the fractions produced from *A. afra* peaked at around 520nm. The whole extract nanoparticle appeared to decreasing from a peak. However, has a slight peak at 520nm and appears to become stable by 580nm.

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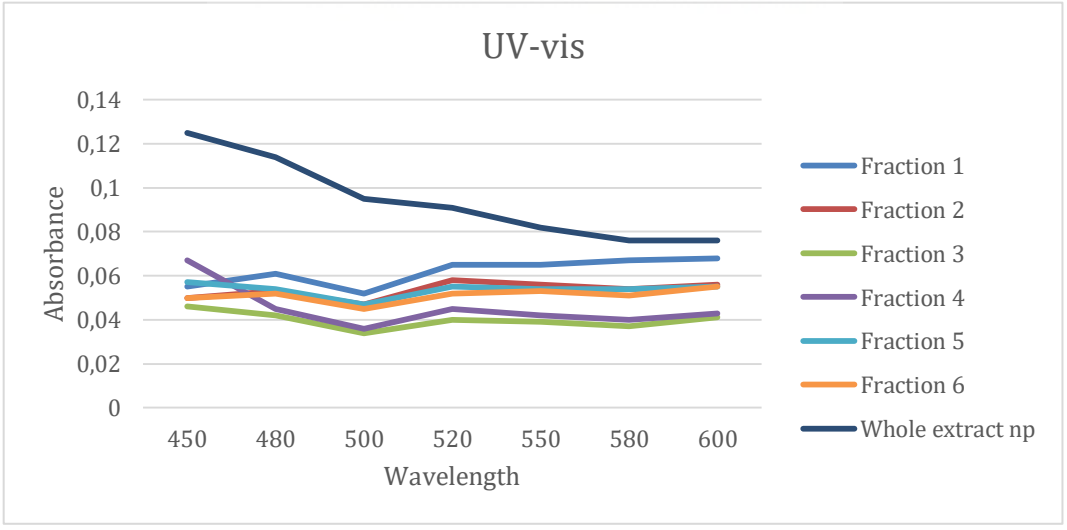

Figure 3.4. UV-vis analysis of nanoparticles produced.

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3.3.2. TEM analysis

The morphology of the gold particles was determined by the TEM images (**Figure 3.5** A–C) and are spherical in shape. As indicated previously by the zeta sizer, particles are polydispersed, however the particles do not vary too greatly in size. Nanoparticles did not show any agglomeration and is a confirmation of their colloidal stability. Nanoparticles agglomerate when having low colloidal stability and result in larger particles (Nazarenus et al. 2014).

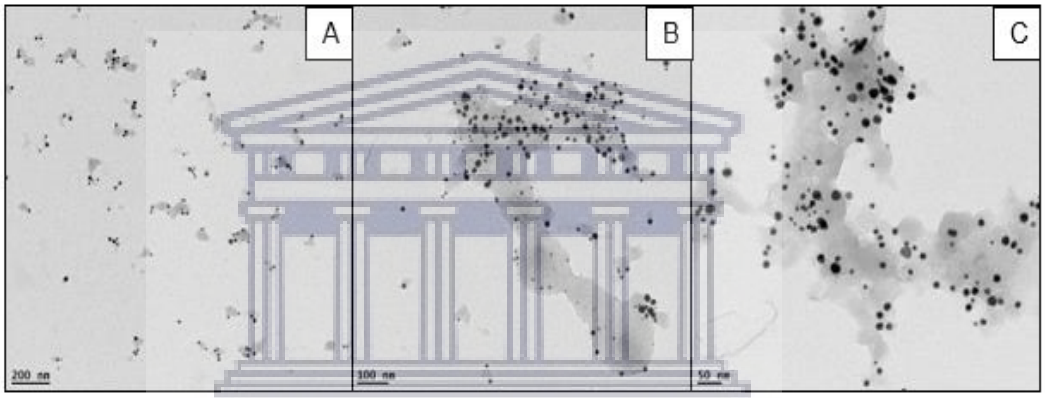

Figure 3.5. TEM images indicating the presence of spherical nanoparticles recorded at various magnifications (A–C).

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3.4. Cytotoxicity

The term toxicity is considered to be rather vague (Fratoddi, 2015). Theoretically, the toxicology of a substance is related to the adverse effects that the particular generic substance exerts on living organisms (Fratoddi, 2015). Furthermore, the cytotoxicity of nanomaterials in known to be largely dependent on the dosage used, particle uptake, colloidal stability, and dissolution among other physiochemical factors (Net et al, 2009). A study conducted by Huang et al. (2012) evaluated the impact of nanoparticles on cell viability and mechanics,

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using silica as a representative NP. In their study, spherical (100 nm), short rod (240 nm length/ 100nm diameter) and long rod (450 nm length/ 100 nm diameter) particles were assessed *in vitro* using A375 human melanoma cells. They concluded that none of the particles showed any signs of toxicity under the standard MTT assay protocol (Huang et al, 2012).

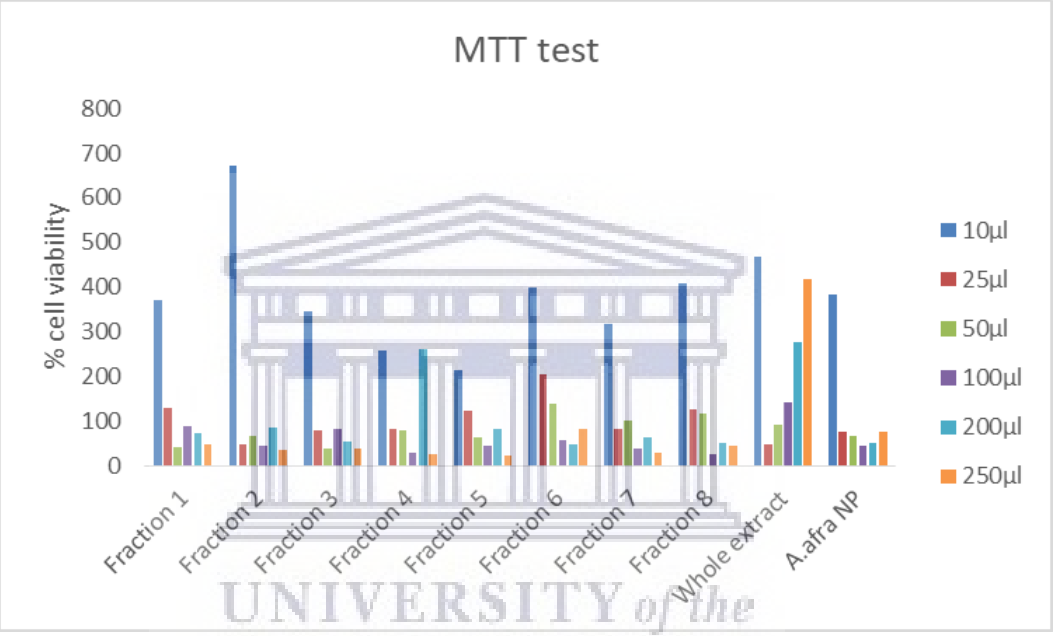

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Figure 3.6. Percentage (%) cell viability of hek293 cells treated with various volumes of gold nanoparticles.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium, also known as MTT, bromide tetrazolium reduction assay was the first homogeneous cell viability assay suitable for high throughput screening developed for a 96-well format (Masmann. 1983). The MTT tetrazolium assay technology is popular in academic laboratories and has been widely adopted proven by thousands of articles published in which the assay is used (Riss, et al. 2013). An MTT

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substrate is typically prepared in a physiologically balanced solution before addition to cells in culture (Riss, et al. 2013). The MTT substrate in solution is typically added at a final concentration between 0.2 - 0.5mg/ml and incubated for a period of 1 to 4 hours (Riss, et al. 2013). The quantity of formazan produced by the cells is presumed to be directly proportional to the number of viable cells within the well (Riss, et al. 2013). This quantity of formazan is measured through the measurement of changes in the absorbance of the solution within the well at 570 nm using a plate reading spectrophotometer (Riss, et al. 2013).

MTT is converted into purple coloured formazan by viable cells with an active metabolism (Riss, et al. 2013). This formazan has an absorbance maximum of around 570nm, hence the reading at the particular wavelength (Riss, et al. 2013). In the case of cell death, the cells lose the ability to convert the MTT to formazan (Riss, et al. 2013). Therefore, the coloured formation produced by the formazan is a convenient and useful marker for only viable cells (Riss, et al. 2013). The exact mechanism of MTT reduction into formazan is, unfortunately, poorly understood, however it is believed to involve a reaction incorporating NADH, or similar reducing molecules, in order to transfer electrons to MTT (Riss, et al. 2013). One particular caveat to consider when measuring the cytotoxicity of nanoparticles is their size. Generally, as AuNPs become smaller, their uptake by cells increases. This higher uptake may result in a higher concentration of AuNPs in the cell, which may lead to cell death as a result of particle accumulation rather than toxicity (Lee et al. 2019).

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The MTT assay (**Figure 3.6)** shows the effects of different amounts of the various nanoparticles produced on HEK293 cells. Gold nanoparticle toxicity is dependent on synthesis method, size, shape, surface coating, surface charge and functionalized molecules (Pan, et al. 2007). However, gold nanoparticles are considered to be non-toxic agents and the overall cytotoxicity of gold nanoparticles is, generally, at an acceptable level (Pan, et al. 2007). A study by Adnan et al. (2016) substantiates that there was no significant toxicity for spherical, rod, or star-shaped gold NPs up to concentrations of 100 μg/mL in MCF-7 human breast adenocarcinoma. This appears to be the case in these nanoparticles as the cell viability does not have a set pattern and most cells appeared to proliferate beyond 100%. At a dosage of 100ng all the cells experienced a high percentage of proliferation, which may indicate that the dosage was too little to have any toxic effects and thus the cells continued to grow normally over the treatment period. Furthermore, at a dosage of 250ng; fractions 1-8, the whole extract and the whole extract nanoparticles all displayed viability percentages of 100% and below. Fractions 1, 5 and 8 showed a percentage of around 100%, indicating that the dosage allowed the growth of the cells to maintain stable. Fractions 2, 3, 4, 7, the whole extract and whole extract nanoparticle showed a decline in percentage. This indicates that at dosages of 250ng and above, for particles produced from these particular fractions, a toxic effect on the cells was exhibited. Likewise, a dosage of 500ng, only fraction 6 had proliferation above 100%, while fractions 7 and 8 were close to 100%. All the other tested nanoparticles and whole extract appeared to show toxicity on the cells tested.

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All the nanoparticle treated cells showed low percentages and it may be considered that the nanoparticles at 1000 ng are toxic to the cells. At 2000ng of treatment, all the cells showed toxicity, with the exception of fraction 4 and the whole extract. Both the whole extract and fraction 4 showed a proliferation of the cells to about 200%. This indicates that those treatments at that volume is not toxic. At 2500ng the only proliferation in cells took place in those treated with the whole extract. The whole extract showed a proliferation of about 400% while all other treated cells showed a massive decrease in viability. This may indicate that the whole extract on its own actually increases cell growth, however in gold nanoparticle form it may be toxic at that volume. These results confirm the established notion that NP-mediated cytotoxicity appears to be governed by several factors: quantity of NP uptake, particle physicochemical properties, particle−cell interactions, and cell type (Kinnaer et al, 2017).

The results indicate a unified dosage that may be used for all treatments. Hence, it was concluded that the treatment did not have a significant effect on the cells, but rather limiting factors (media degradation due to cell metabolism) during normal cell growth interfered prohibited cell proliferation.

3.5. **Glucose Uptake**

The present study used an integrated omics-based approach to investigate NP-induced glucose metabolic function. Evaluation of glucose uptake in mammalian

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tissues may seem a very basic step, however, it is imperative to identify the hypoglycemic effect of plant extracts (Yeong and Ardith, 2009).

Given the belief that reductions in the amount of intracellular glucose are caused by increased uptake efficiency, a detailed description of the mechanism used to measure glucose uptake was deemed necessary to fully understand the significance of the findings presented herein. When 2-deoxyglucose (2DG) is added to cells, it is transported across the membrane and rapidly phosphorylated in the same manner as glucose. However, enzymes that further modify glucose-6-phosphate (G6P) cannot modify 2DG6P, and thus this membrane-impermeable analyte accumulates in the cell. After a brief period of incubation, the acidic Stop Buffer is added to lyse cells, terminate uptake and destroy any NADPH within the cells. A high-pH buffer solution (Neutralization Buffer) is then added to neutralize the acid. A Detection Reagent is added to the sample wells.

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Glucose-6-Phosphate Dehydrogenase oxidizes the deoxyglucose to 6-phosphodeoxygluconate and simultaneously reduces NADP+ to NADPH. The Reductase uses NADPH to convert the proluciferin to luciferin, which is then used by Ultra-Glo™ Recombinant Luciferase to produce a luminescent signal that is proportional to the concentration of 2DG6P.

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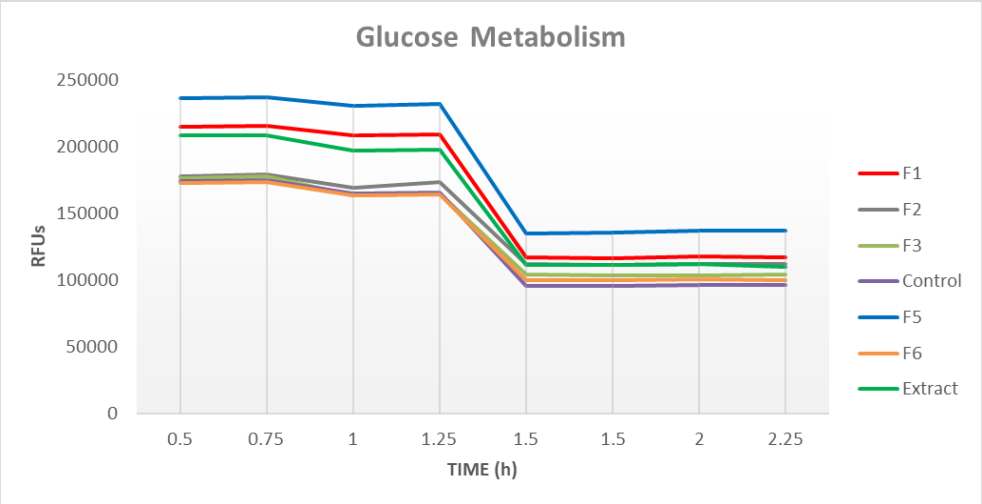

Figure 3.7. Graph depicting relative glucose uptake via glucose metabolism of nanoparticle treated HEK293 cells over time.

Figure 3.7 shows HEK293 cells treated with the various nanoparticles synthesized and the glucose uptake exhibited by the cells over time. Relative Fluorescent Units (RFUs) are directly proportional to the amount of metabolized glucose, and thus, confer an indication of the amount of glucose taken up and by the cells. Hence, the glucose uptake is measured by the rate of metabolism of the glucose by the cells, given by the area under the curve. The control (untreated HEK293 cells) was used to generate a baseline reading for glucose uptake. In reference to the figure above, the whole extract nanoparticles produced a definite overall increase in glucose uptake when compared to the control. In fact, it was also observed that all nanoparticle treated cells exhibited an increased glucose metabolism after a given time period.

The first reading of the glucose uptake assay was recorded at 0,5 hours, allowing the initial surge in glucose uptake and metabolism following starvation to

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normalize. Every instance of treated cells appeared to have a slight rise in glucose uptake from their initial point of recording. The cells then had a slight decrease at 0,75 hours as saturation is reached, before increasing again at 1 hour. For the control cells, their maximum glucose metabolism occurred around 1,25 hours. Between 1,25 hours and 1,5 hours fraction 1, fraction 2, fraction 3, fraction 4, fraction 5, fraction 6, the whole extract nanoparticles and the control cells experienced a steady decrease in glucose metabolism.

The hepatocytes appeared to reach a stable rate of glucose metabolism at 1,5 hours and continued to metabolize glucose around this rate for the remainder of the assay. Fractions 2, 3, 6 and the control cells had the same initial rate of glucose uptake. Fraction 2 has the slight increase in glucose uptake between 0,75 and 1 hour and maintains this rate until the 1.5-hour mark. However, fractions 3,6 and the control appeared to slowly decrease over the same time period, before reaching a plateau at 1.5 hours.

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Once the rate of all the cells started decreasing at 1,25 hours, fraction 2 had a slower decrease than fractions 3,6 and the control, eventually reaching the same rate as the whole extract nanoparticle. The cells with the lowest glucose metabolism rate recorded were the control cells. Fractions 3 and 6 were just above the control cells, however, they initially had the same rate of glucose uptake. The cells that exhibited the highest rate of glucose uptake between initiation and the completion of the assay were those treated with nanoparticles synthesized from fraction 5.

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From the initiation phase, through the decreasing phase and final phase of the assay, the F5 cells consistently had a higher rate of glucose metabolism than that of the other cells (**Figure 3.7**). The cells treated with fraction 1 and the whole extract nanoparticles both had high initial glucose metabolism rates, though lower than those treated with the nanoparticles synthesized from fraction 5. Between 0,75 hours and 1 hour the cells treated with the whole extract nanoparticle showed a larger decline in the glucose uptake rate than that of the cells treated with fraction 1 nanoparticles. The final rate of glucose metabolism for the cells treated by fraction 1 synthesized nanoparticles were higher than the whole extract synthesized nanoparticles.

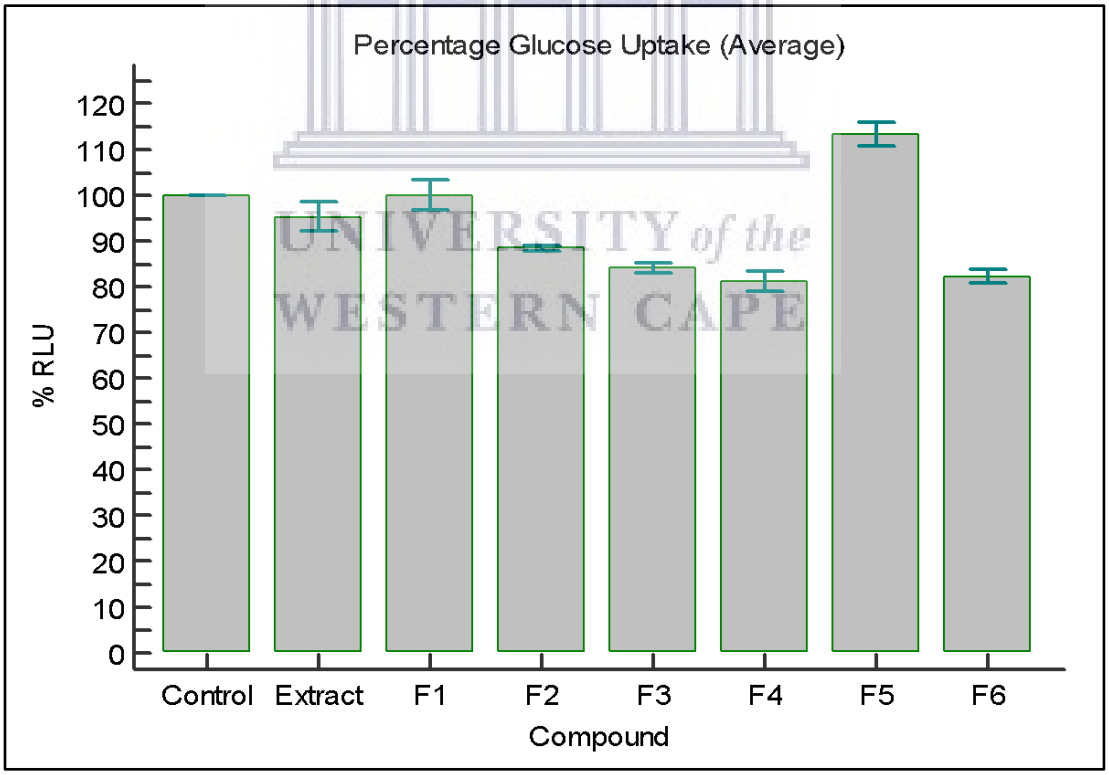

Figure 3.8. Percentage of average glucose metabolism over 2,25 hours for cells treated with nanoparticles synthesized from the various fractions of *A. afra*.

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Statistical analysis was performed on the average glucose uptake and metabolism (**Figure 3.8**). This ensures an accurate representation of the total glucose taken up and metabolized by the cells. It was determined that cells treated by the nanoparticles synthesized from fraction 5 of the *A. afra* extract had the highest percentage of glucose taken up and metabolized. Second, were the cells treated with nanoparticles synthesized from fraction 1, closely followed by the control cells. The results generated here indicates that the most effective nanoparticles were those synthesized from fraction 5.

Moreover, nanoparticles produced by the whole *A. afra* extract and fractions 2, 3, 4 and 6 displayed a lower total glucose uptake than the control cells. This may suggest that those nanoparticles have an unrelated biological function and would not aid in glucose uptake and metabolism. The cells treated with whole *A. afra* extract and fractions 2, 3, 4 and 6 caused a reduction in glucose uptake and metabolism. Therefore, it may be suggested that the compounds within those fractions may hinder glucose uptake and metabolism, although further investigation will be required to confirm these findings.

In the present study, the nanoparticles produced from fraction 5 were the most successful and it may be assumed that the native compounds within fraction 5 are the most effective at improving glucose uptake and metabolism. A closer analysis was done on fractions 1 and 5, compared to the control and whole extract cells (**Figures 3.9 and 3.10**). Comparing the glucose uptake between the

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control cells and those treated with nanoparticles produced from fraction 1 yielded a p-value of 1.

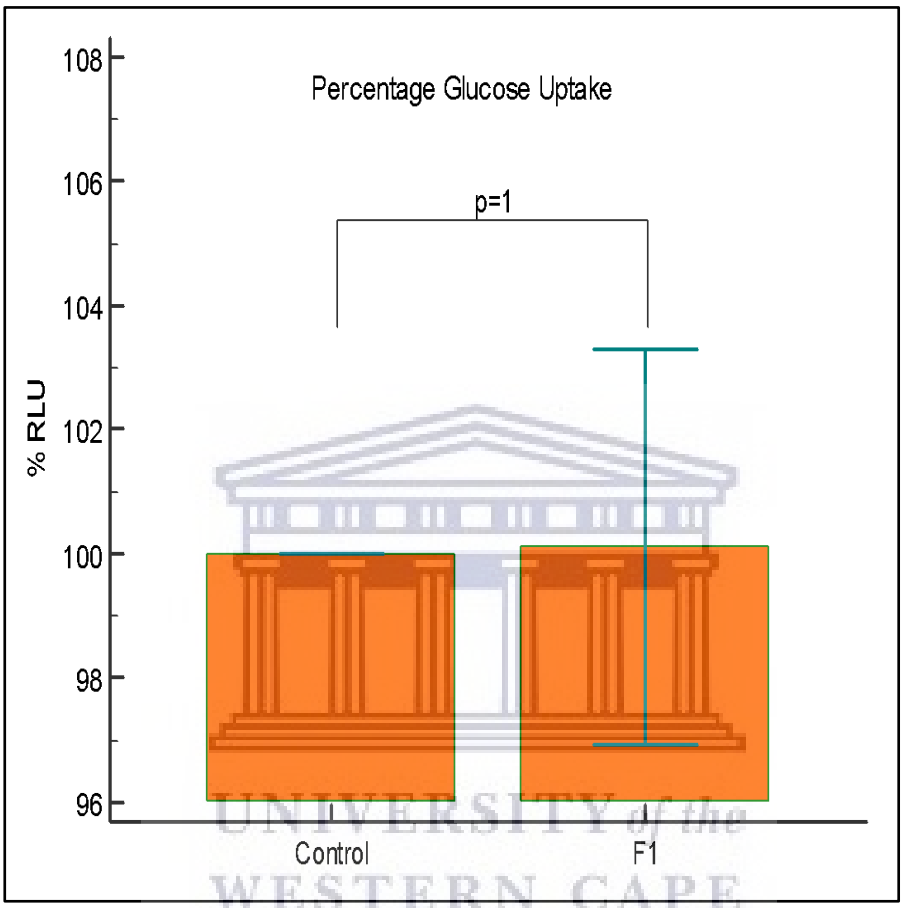

Figure 3.9. Statistical analysis comparing the control cells with those treated with nanoparticles synthesized using fraction 1.

However, analysis between the cells treated with the whole extract nanoparticle and those treated with nanoparticles synthesized from fraction 1 showed a larger difference in percentage glucose uptake. Nevertheless, a p-value of 0.0927 was calculated, indicating that no significant glucose uptake and metabolism was observed.

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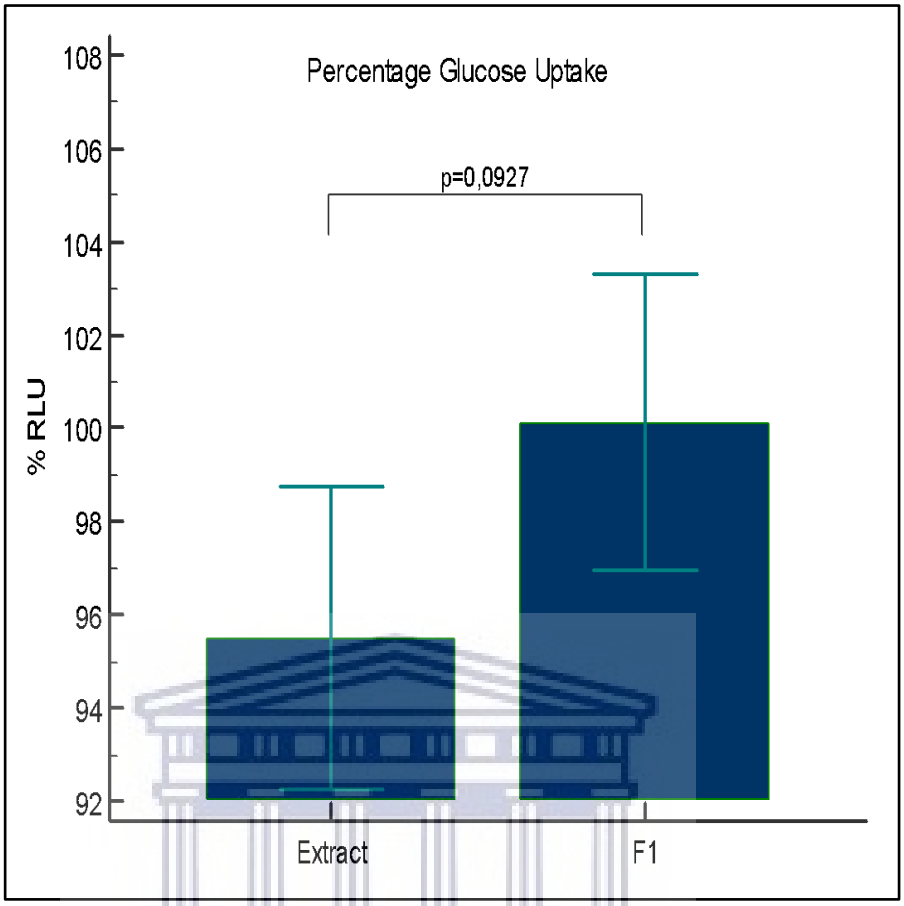

Figure 3.10. Statistical analysis comparing the cells treated with whole extract to those treated with nanoparticles synthesized using fraction 1.

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The same statistical analysis was applied to the cells treated with nanoparticles synthesized from fraction 5 (F5) and the whole extract nanoparticle treated cells, as well as the control (**Figures 3.11 and 3.12**). Between the control cells and those treated with fraction 5 nanoparticles, p=0.0005 was calculated. This indicated significant glucose uptake and metabolism in those treated with fraction 5 nanoparticles when compared to the control.

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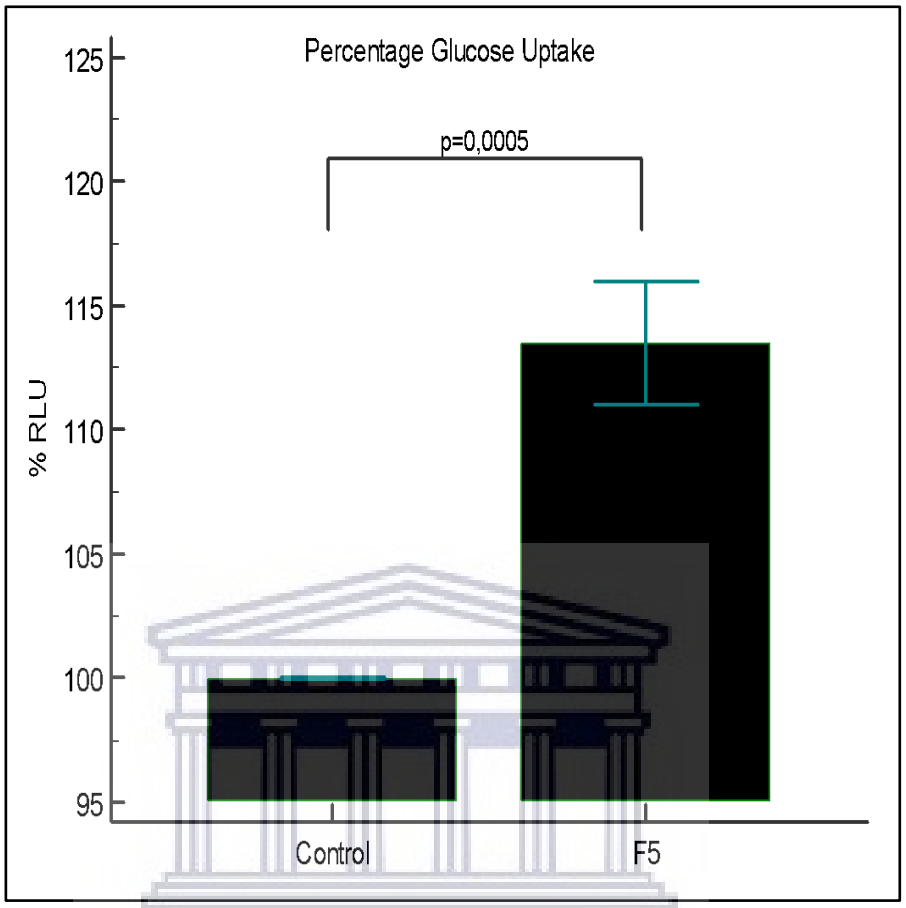

Figure 3.11. Statistical analysis comparing the control cells with those treated with nanoparticles synthesized using fraction 5.

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Furthermore, the analysis of fraction 5 nanoparticle treated cells compared to the whole extract nanoparticle treated cells, a p-value of 0.0008 was calculated. This too indicated significant glucose uptake and metabolism by the fraction 5 nanoparticle treated cells, compared to the whole extract nanoparticle treated cells. In addition, no hemolysis was observed in all the concentrations tested during the glucose uptake assay. Further studies may focus on identifying mechanism of action of these particular NPs in glucose uptake.

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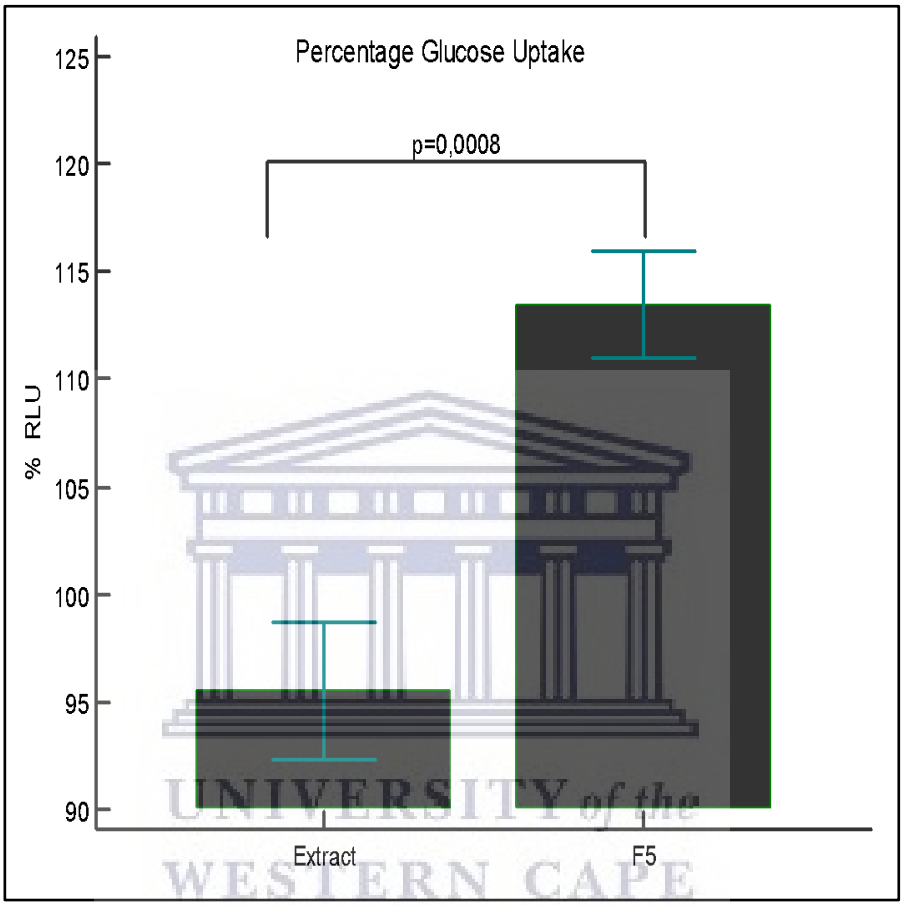

Figure 3.12. Statistical analysis comparing the cells treated with whole extract to those treated with nanoparticles synthesized using fraction 5.

3.6. Study Prospects

With further studies, the application of nanoparticles could become an integral part in drug administration. In this study it has been experimentally shown that phytotherapeutic nanoparticles of the right size and stability are effortlessly taken up by mammalian cells and may, therefore, present an innovative

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opportunity in drug discovery and delivery. In addition, specific tissues could be directly targeted and with further research, toxicity and adverse events could be reduced. Furthermore, use of nanoparticles as a drug delivery system may decrease the clearance time of the vehicle of delivery as cells may expel the nanoparticles once the medication has been delivered more rapidly. The fast delivery action of the nanoparticles may potentially result in faster clearance of drug metabolites, from the body.

Advances such as those investigated in this study could lead to an alternative medicine source for patients suffering, not only from type 2 diabetes, but from various diseases and medical conditions. It may serve as both an aid to conventional medicine, as well as an alternative. Though it may not eliminate the possibility of adverse side effects, phytotherapeutics may reduce the number and frequency of adverse side effects associated with conventional drugs.

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Herbal medicine is traditionally used in many cultures around the world and is believed to be the most cost-effective method for many people. A plant-based treatment would appeal to people who believe in the traditional herbal medication over the chemically synthesized medications. An effective plant treatment, whether stand-alone or in combination treatment, will likely be more cost effective than conventional medicine, hence, availability and access to treatment may be infinitely improved. Moreover, herbal medicine has been proven to have an effect on a specific disease, such as the case of *A. afra* in type 2 diabetes. Coupled with the effectiveness of nano-based delivery in a system

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that ensures the slow release of the phytochemical, may result in a decrease in adverse side effects often caused by use of the *A. afra* plant itself.

Furthermore, it has been suggested that the development of an effective plant treatment may by-pass numerous genetic factors associated with conventional drug treatment. However, numerous studies will be required to amass a body of evidence large enough to prove or disprove this notion.

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CHAPTER 4

4. Study Limitations

This study has shown that gold nanoparticles may be successfully produced from *A. afra*, as well as the fractions produced through HPLC. Green synthesis of nanoparticles is often difficult to optimize as nanoparticle specifications are difficult to control. Unavoidably, the *A. afra* plant and the constituents of the fractions proved to be relatively time-consuming to optimize and produce nanoparticles of sizes below 150nm in diameter, as well as within the required polarity (mV). However, the nanoparticles produced proved to be stable and were charges were consistently measured to be approximately -18mV.

The cytotoxicity of the nanoparticles could not be accurately determined and it may be concluded that the plant extract as well as the gold nanoparticles have a very low cytotoxicity towards HEK293 cells. At 100ng of treatment it may even increase proliferation of cells but further investigation may be required to determine its effectiveness at this concentration. Further tests were conducted on the same cell line using 1000ng of nanoparticles and no significant cell death occurred. This indicates that human error or ineffective treatment of the cells during the MTT assay may have influenced the results.

With regards to the glucose uptake, F5 nanoparticle-treated cells had a significant increase in glucose uptake compared to the control and whole extract

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nanoparticle treated cells. This indicates that the bioactive ingredient with the most significant anti-diabetic effects is present in fraction 5. This has narrowed down the constituents of *A. afra* with the desired effects to those within fraction 5. However, due to time constraints within the project, the precise phytochemical responsible for improving glucose uptake could not be identified. Although, this will be investigated in future work, in addition to an evaluation of the role of insulin with these phytochemicals.

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CHAPTER 5

5. Conclusion and Recommendations

Current research classifies Type 2 diabetes as most prevalent non-communicable diseases in South. Metformin, a first-line drug in the treatment of Type 2 diabetes, has been shown to have an efficacy rate of 43% due to poor drug uptake and metabolism. Furthermore, herbs commonly used traditional medicine have shown promise in the treatment of Type 2 diabetes.

This study has shown that bioactive compounds useful in the treatment of type 2 diabetes may be isolated from *A. afra*. Furthermore, a nano-carrier formulation was produced from these compounds and evaluated in this regard. Stable gold nanoparticle production was observed in this study and it was demonstrated that the exogenous particles were small enough to be transported across the mammalian cell wall.

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In addition, nanoparticles produced from fraction 5 proved to have a positive effect on glucose uptake *in vitro*. Moreover, these gold nanoparticles also exhibited no toxic effects on the HEK293 cells within the tested dosages. It may, therefore, be concluded that the constituents of *A. afra* present an innovative route for the treatment of type 2 diabetes in patients with a decreased response to Metformin. However, further studies are required to determine a toxic dosage

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of these gold nanoparticles *in vitro* and to successfully isolate and identify the exact phytochemical responsible for the improved glucose uptake.

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References

Abdulfatai B. Olokoba, Olusegun A. Obateru, Lateefat B. Olokoba. (2012) Type 2 Diabetes Mellitus: A Review of Current Trends. *Oman Medical Journal*. Vol. 27, No. 4. Pages 269-273.

Adnan, N. N. M.; Cheng, Y. Y.; Ong, N. M. N.; Kamaruddin, T. T.; Rozlan, E.; Schmidt, T. W.; Duong, H. T. T.; Boyer, C. Effect of Gold Nanoparticle Shapes for Phototherapy and Drug Delivery. Polym. Chem. 2016, 7, 2888−2903.

Agnihotri J, Saraf S, Khale A. (2011) Targeting: new potential carriers for targeted drug delivery system. *International Journal of Pharmaceutical Sciences Review and Research.* Volume 8. Page 117.

Anderson Z. L, Scopelliti E. M, Trompeter J. M, Havrda D. E. (2015) Management of prediabetes: a comparison of the treatment approaches utilized by a family practice clinic and an internal medicine/endocrinology practice. *Journal of Pharmacy Practice.* Volume 28(1), Pages 86-92.

Aromal, S. A., Philip, D. (2012) Green synthesis of gold nanoparticles using Trigonella foenum-graecum and its size dependent catalytic activity. *Spectrochim*. Act 97. Pages 1–5.

Bhambere D, Gaidhani K, Harwalkar M, Nirgude P. (2015). LYOPHILIZATION / FREEZE DRYING – A REVIEW. *World Journal of Pharmaceutical Research.* Volume 4. Pages 516-543.

Bird I M. (1989) High performance liquid chromatography: Principles and clinical applications. *British medical journal (Clinical research edition).* Volume 299. Pages 783-787.

Bodeker C., Bodeker G., Ong C. K., Grundy C. K., Burford G., Shein K. (2005). *WHO* Global Atlas of Traditional, Complementary and Alternative Medicine. Geneva, Switzerland: World Health Organization.

UNIVERSITY of the WESTERN CAPE

http://etd.uwc.ac.za/

Boylan J, Nail S. (2002) Parental Products, in: Banker, G.S., Rhodes, C.T. (Eds.), *Modern Pharmaceutics*. Marcel Dekker, Inc, New York. Pages 576–625.

Braünlich, P. M. et al. (2018). Polysaccharides from the Southern African medicinal plant Artemisia afra: structure and activity studies. *Fitoterapia*. Volume 124. Pages 182 –187.

Burits M, Asres K, Bucar F (2001) The antioxidant activity of the essential oils of Artemisia afra, Artemisia abyssinica and Juniperus procera. *Phytotherapy Research*. Volume 15(2). Pages 103– 108.

Chiniwala N and Jabbour S (2011) Review Management of diabetes mellitus in the elderly. *Curr Opin Endocrinol Diabetes Obes*. Volume18(2). Pages 148-52.

Choi, C. H., Zuckerman, J. E., Webster, P., & Davis, M. E. (2011). Targeting kidney mesangium by nanoparticles of defined size. *Proceedings of the National Academy of Sciences of the United States of America*. Volume *108*(16). Pages 6656–6661.

UNIVERSITY of the
WESTERN CAPE

Clayton, K. N., Salameh, J. W., Wereley, S. T., & Kinzer-Ursem, T. L. (2016). Physical characterization of nanoparticle size and surface modification using particle scattering diffusometry. *Biomicrofluidics*. Volume *10*(5).

Coetzee C, Jephtas E, Reinten E. (1999) Indigenous plant genetic resources of South Africa. *Perspectives on new crops and new uses ASHS Press*. Pages 160-136.

Collier C. A, Bruce C. R, Smith A. C, Lopaschuk G, Dyck D. J. (2006) Metformin counters the insulin-induced suppression of fatty acid oxidation and stimulation of triacyglycerol storage in rodents' skeletal muscle. *American*

http://etd.uwc.ac.za/

Journal of Physiology Endocrinology Metabolism. Volume 219(1). Pages 182-189.

Cox E. M and Elelman D. (2009) Test for screening and diagnosis of type 2 diabetes. *Clinical Diabetes.* Volume 4(27). Pages 132-138.

Cunningham A, et al. (1996) Zulu Medicinal Plants: An inventory. South Africa, University of Natal press. Scottsville. Page 327.

Dabe, N. E., & Kefale, A. T. (2017). Antidiabetic Effects of Artemisia Species: A Systematic Review. *Ancient science of life*. Volume *36*(4). Pages 175–181.

Dahiru T. (2008). P - value, a true test of statistical significance? A cautionary note. *Annals of Ibadan postgraduate medicine*. Volume *6*(1). Pages 21–26.

Danaei M, Dehghankhold M, Ataei S, Davarani F, Javanmard R, Dokhani A, Khorasani S, Mozafari M (2018) Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. *Pharmaceutics.* Volume 10. Page 57.

UNIVERSITY of the
WESTERN CAPE

Dube, A., 2006. The design, preparation and evaluation of Artemisia afra and placebos in tea bag dosage form suitable for use in clinical trials. MSc Dissertation. University of the Western Cape, Bellville.

Ekor M. (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in pharmacology*. Volume 4. Article 117.

Eshetu N, Afework M, Makonnen E, Debella A, Ergete W, Tolesssa T. (2016) Evaluation of the Acute and Sub-chronic Toxic Effects of Aqueous Leaf Extracts

http://etd.uwc.ac.za/

of Artemisia afra on Liver, Kidney and Some Blood Parameters in Wistar Rats. *Advances in Bioscience and Bioengineering*. Vol. 1. No. 1. Pages 1-9.

Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. (1997) Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care.* Volume 20. Pages 1183–1197.

Francis, S., Joseph, S., Koshy, E. P. (2017). Green synthesis and characterization of gold and silver nanoparticles using Mussaenda glabrata leaf extract and their environmental applications to dye degradation. *Environmental Science and Pollution Research.* Volume 24. Page 17347.

Fratoddi, I., Venditti, I., Cametti, C. et al. Nano Res. (2015) How toxic are gold nanoparticles? The state-of-the-art. *Nano research*. Volume 8. Issue 6. Pages 1771-1799.

Fujioka K (2007) Review Pathophysiology of type 2 diabetes and the role of incretin hormones and beta-cell dysfunction. *JAAPA*. Pages 3-8.

Ghazanfar K, Ganai B. A, Akbar S, Mubashir K, Dar S. A, Dar M. Y, Tantry M. A (2014) Antidiabetic activity of Artemisia amygdalina Decne in streptozotocin induced diabetic rats. *Biomed Res Int*. Page 185676.

Global burden of diabetes. (2011) International Diabetes federation. Diabetic atlas fifth edition. Brussels.

Graven E, Deans S, Mavi S, Gundidza M. G. and Svoboda K. P. (1992). Antimicrobial and antioxidative property of the volatile (essential) oil of Artemisia afra Jacq.. *Flav. Frang. J.* Volume 7. Pages 121–123.

Graven E. H, Webber L, Venter M, Gardner J. B. (1990) The development of Artemisia afra (Jacq.) as a new essential oil crop. Journal of Essential Oil Research. Volume 2. Pages 215-220.

http://etd.uwc.ac.za/

Halban P. A, Polonsky K. S, Bowden D. W, Hawkins M. A, Ling C, Mather K. J, Powers A. C, Rhodes C. J, Sussel L, Weir G. C. (2014) β-cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. *Diabetes Care*. Volume 37. Pages 1751-1758.

Hilliard O. M. (1977) Compositae in Natal. University of Natal Press, Pietermaritzburg. Pages 360–361.

Hussain, Imtiyaz & Singh, N.B. & Singh, Ajey & Singh, Himani & Singh, Subhash. (2016). Green synthesis of nanoparticles and its potential application. *Biotechnology Letters.* Volume 38. Pages 545-560.

International Expert Committee. (2009) International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care*. Volume 32. Pages 1-8.

Issa I and Bule M H (2015). Hypoglycemic Effect of Aqueous and Methanolic Extract of Artemisia afra on Alloxan Induced Diabetic Swiss Albino Mice. *Evidence-Based Complementary and Alternative Medicine.* Volume. 2015. Page 5.

Khaled, J. M., Alharbi, N. S., Kadaikunnan, S. (2017) Green Synthesis of Ag Nanoparticles with Antibacterial Activity Using the Leaf Extract of an African Medicinal Plant, Ipomoea asarifolia (Convolvulaceae). *Journal of Cluster Science.* Volume 28. Page 3009.

http://etd.uwc.ac.za/

Khan I, Saeed K, Khan I. (2017) Nanoparticles: Properties, applications and toxicities. *Arabian jounrnal of chemistry*.

Kharroubi AT, Darwish HM. (2015) Diabetes mellitus: The epidemic of the century. *World Journal of Diabetes*. Volume 6(6). Pages 850-867.

Kim Y. D, Park K. G, Lee Y. S, Park Y. Y, Kim D. K, Nedumaran B, Jang W. G, Cho W. J, Ha J, Lee I. K, Lee C. H, Choi H. S. (2008) Metformin inhibits hepatic gluconeogenesis through AMP-activated protein kinase-dependent regulation of the orphan nuclear receptor SHP. Diabetes. Volume 57(2). Pages 306-14.

Kinnear, C., Moore, T. L., Rodriguez-Lorenzo, L., Rothen-Rutishauser, B. and Petri-Fink, A. (2017). Form Follows Function: Nanoparticle Shape and Its Implications for Nanomedicine. *Chemical Reviews*, 117**,** 11476-11521.

Komperlla M. K (2004) The formulation and evaluation of rapid release tablets manufactured from Artemisia afra plant material. M. Pharm. Thesis, University of the Western Cape, Bellville, South Africa.

Korkmaz H and Gürdal A (2002) Effect of Artemisia santonicum L. on blood glucose in normal and alloxan-induced diabetic rabbits. *Phytother Res.* Volume 16(7). Pages 675-6.

Kraft, J. C., Freeling, J. P., Wang, Z., & Ho, R. J. (2014). Emerging research and clinical development trends of liposome and lipid nanoparticle drug delivery systems. *Journal of pharmaceutical sciences*. Volume *103*(1). Pages 29–52.

UNIVERSITY of the WESTERN CAPE

http://etd.uwc.ac.za/

Kuppusamy P, Yusoff M, Maniam G, Govindan N. (2016) Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report. *Saudi Pharmaceutical Journal.* Volume 24. Issue 4. Pages 473-484.

Lee, Y. J., Ahn, E. Y., & Park, Y. (2019). Shape-dependent cytotoxicity and cellular uptake of gold nanoparticles synthesized using green tea extract. *Nanoscale research letters.* Volume *14*(1). Page 129.

Li Y, Zheng M, Zhai X, Huang Y, Khalid A, Malik A. (2015) Effect of- *Gymnema Sylvestre Citrullus colocynthis* and *Artemisia absinthium* on blood glucose and lipid profile in diabetic human. *Acta Pol Pharm*. Volume 72. Pages 981–5.

Lim T. Y, Poole R. L, Pageler, N. M. (2014) Propylene glycol toxicity in children. *Journal of Pediatric Pharmacology Therapy.* Volume 19. Pages 277–282.

Liu N, Van der Kooy F, Verpoorte R. (2009) Artemisia afra: A potential flagship for African medicinal plants?, *South African Journal of Botany*. Volume 75. Issue 2. Pages 185-195.

Mander, M. (1998) Marketing of Indigenous Medicinal Plants in South Africa: A Case Study in KwaZulu-Natal. Food and Agricultural Organization of the United Nations, Rome.

Mayfield J. A and White R. D. (2004) Insulin therapy for type 2 diabetes: rescue, augmentation, and replacement of beta-cell function. *Am Fam Physician.* Volume 70(3). Pages 489-500.

http://etd.uwc.ac.za/

Mishra P, Stringer M. D. (2010) Sciatic nerve injury from intramuscular injection: a persistent and global problem. *Int. J. Clin. Pract*. Volume 64. Pages 1573–1579.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*. Volume 65. Pages 55–63.

Mukinda J. T (2005) Acute and chronic toxicity of the flavonoid- containing plant, Artemisia afra in rodents, Thesis, University of the Western Cape.

Mwangi J. W, Anchola J. K, Sinei K. A, Lwande W, Laurent R. (1995) Essential oil constituents of Artemisia afra Wild. *Journal of Essential Oil Research.* Volume 7. Pages 97–99.

Nadeem M, Abbasi B, Younas M, Ahmad W, Khan T (2017) A review of the green syntheses and anti-microbial applications of gold nanoparticles. *Green Chemistry Letters and Reviews.* Volume 10(4). Pages 216-227.

UNIVERSITY of the
WESTERN CAPE

Nazarenus M, Zhang Q, Soliman M G, del Pino P, Pelaz B, Carregal-Romero S, Rejman J, Rothen-Rutishauser B, Clift M J D, Zellner R, Nienhaus G U, Delehanty J B, Medintz I L, Parak W J. (2014) In vitro interaction of colloidal nanoparticles with mammalian cells: What have we learned thus far?. *Beilstein Journal of Nanotechnology*. Volume 5. Pages 1477-1490.

Nel, A. E.; Madler, L.; Velegol, D.; Xia, T.; Hoek, E. M.; Somasundaran, P.; Klaessig, F.; Castranova, V.; Thompson, M. Understanding Biophysicochemical Interactions at the Nano-Bio Interface. Nat. Mater. 2009, 8, 543−557.

Nozuri M (2014) Biosynthesis of gold nanoparticles using plant extracts. *Bioprocess and Biosystems engineering*. Volume 38. Issue 1. Pages 1-14.

http://etd.uwc.ac.za/

Ogurtsova K, da Rocha Fernandes J. D, Huang Y, Linnenkamp U, Guariguata L, Cho N. H (2017) IDF diabetes atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Research Clinical Practice*. Volume 128. Pages 40–50.

Øie S, Benet L. (2002) The effect of Route Administration and Distribution on Drug Action, in: Banker, G.S., Rhodes, C.T. (Eds.), *Modern Pharmaceutics.* Marcel Dekker, Inc, New York, Pages 187–214.

Oyedeji A. O, Afolayan A. J, Hutchings A (2009) Compositional variation of the essential oils of Artemisia afra Jacq. from three provinces in South Africa – a case study of its safety. Nat. Prod. Commun. Volume 4 (6). Pages 849–852.

Pan Y, Neuss-Stein S, Leifert A, Fischler M, Wen F, Simon U, Schmid G, Brandau W, Jahnen-Dechent, W. (2007) *Size-Dependent Cytotoxicity of Gold Nanoparticles. Small.* Volume 3. Pages 1941-9.

Patil GV, Dass SK, Chandra R (2011) Artemisia afra and Modern Diseases. *Journal Pharmacogenomics and Pharmacoproteomics*. Volume 2. Page 105.

Rasheed T, Bilal M, Iqbal HMN, Li C. (2017) Green biosynthesis of silver nanoparticles using leaves extract of Artemisia vulgaris and their potential biomedial applications. Colloids and surfaces B: Biointerfaces. Volume 158. Pages 408 - 415.

Reddy P D, Swarnalatha D. (2010) Recent Advances in Novel Drug Delivery Systems. *International Journal of PharmTech Research*. Volume 2. Number 3. Pages 2025-2027.

UNIVERSITY of the
WESTERN CAPE

http://etd.uwc.ac.za/

Fontes Ribeiro C. A. (2005) Influence of food and drugs on the bioavailability of antiepileptic drugs. Antiepileptic Drugs: Combination Therapy and Interactions - Google Books. Cambridge Press, Cambridge. Pages 93– 110.

Riss T, Moravec R, Niles A, Duellman S, Benink H, Worzella T, Minor L (2013) Cell Viability Assays. Assay Guidance Manual.

Roberts M. (1990) Indigenous healing plants. *Southern Book Publishers*, Halfway House, Pages 226–228.

Ruiz M & Scioli Montoto S. (2018) Routes of Drug Administration: Dosage, Design, and Pharmacotherapy Success. *ADME Processes in Pharmaceutical sciences*. Pages 97-133.

Sharma B, Crist R. M. & Adiseshaiah, P.P (2017) Nanotechnology as a Delivery Tool for Precision Cancer Therapies. *The AAPS Journal*. Volume 19. Page 16320.

Sharma I, Yngard R, Lin Y. (2009) Silver nanoparticles: Green synthesis and their antimicrobial activities, *Advances in Colloid and Interface Science*, Volume 145, Issues 1–2, Pages 83-96.

Scheen A. J. (2005) Drug interactions of clinical importance with antihyperglycaemic agents: an update. *Drug Safety. Volume* 28(7). Pages 601-631.

Shukla, A. K. & Iravani, S. (2017) Metallic nanoparticles: green synthesis and spectroscopic Characterization. *Environ Chem Lett*. Volume 15. Page 223.

UNIVERSITY of the WESTERN CAPE

http://etd.uwc.ac.za/

Si S, Pal A, Mohanta J, Satapathy S. (2017). Gold Nanostructure Materials in Diabetes Management. *Journal of Physics D: Applied Physics*. Volume 50. Number 13.

Silbernagel, E., Spreitzer, H. and Buchbauer, G. (1990). Non-volatile constituents of Artemisia afra. *Monatsch Chem.* Volume 121(5). Pages 433–436.

Spies L, Koekemoer T. C, Sowemimo A. A, Goosen E. D, van de Venter M, Caspase- dependent apoptosis is induced by Artemisia afra Jacq. ex Willd in a mitochondria- dependent manner after G2/M arrest. *South African Journal Botany*. Volume 84. Pages 104– 109.

Tahraoui A, El-Hilaly J, Israili ZH, Lyoussi B (2007) Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in South-Eastern Morocco (Errachidia province). *J Ethnopharmacol*. Volume 110. Pages 105–17.

Van Wyk B. (2011) The potential of South African plants in the development of new medicinal products. *South African Journal of Botany*. Volume 77. Issue 4. Pages 812-829.

UNIVERSITY of the
WESTERN CAPE

Van Wyk, B. E. (2008). A broad review of commercially important southern African medicinal plants. *Journal of Ethnopharmacology* Volume 119. Pages 342–355.

Van Wyk B. E, Van Oudtshoorn B. Gerike N. (1997) Medicinal Plants of South Africa. *Briza Publications*, Pretoria. Page 142.

Van Wyk B. E, Van Oudtshoorn. B, Gericke N. (2000) Medicinal plants of South Africa. 2nd Ed. *Briza Publications*, Pretoria, South Africa.

http://etd.uwc.ac.za/

Van Wyk, B. E, Van Oudtshoorn B, Gericke, N. (2002) Medicinal Plants of South Africa, 2nd ed. *Briza Publications*, Pretoria, South Africa.

Verma P, Thakur A.S, Deshmukh K, Dr. Jha A.K, Verma S. (2010) ROUTES OF DRUG ADMINISTRATION. *International Journal of Pharmaceutical Studies and Research.* Volume 1. Pages 54 – 59.

Viljoen A. M, Van Vuuren S. F, Gwebu T, Demirci B, Baser K. and Husnu C (2006). The geographical variation and antimicrobial activity of African wormwood (Artemisia afra Jacq.) essential oil. *J.Ess*. Oil Res. Volume 18. Pages 19–25.

Von Koenen E. (2001) Medicinal, poisonous and edible plants in Namibia, *Klaus Hess Publishers*, Windhoek – Göttingen.

Watt, J., Breyer-Brandwijk, M. G., 1932. The medicinal and poisonous plants of southern and eastern Africa. Livingstone, Edinburgh.

Wild S, Roglic G, Green A, Sicree R, King H (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care.* Volume 27(5). Pages 1047-53.

.

.

World Health Organization. Global Report on Diabetes. Geneva: WHO; 2016.

World Health Organization. (1999) Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Geneva: WHO Department of Noncommunicable Diseases Surveillance; 1999.

World Health Organization. (2004). WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems. Geneva, Switzerland: World Health Organization

http://etd.uwc.ac.za/

Yeh Y. C, Creran B, & Rotello, V. M (2012). Gold nanoparticles: preparation, properties, and applications in bionanotechnology. *Nanoscale*. Volume 4(6). Pages 1871–1880.

Yeong, R., Ardith B. (2009) Flaxseed lignin increased glucose uptake by human red blood cells. *Open Nutraceuticals J*. Volume 2. Pages 81-85.

Zhu Y and Zhang C. (2016) Prevalence of Gestational Diabetes and Risk of Progression to Type 2 Diabetes: A Global Perspective. *Current diabetes reports*. Volume 16(1). Page 7.

http://etd.uwc.ac.za/