ANTIOBESITY AND CAECAL MICROBIOME EFFECTS OF DICHLOROMETHANE – METHANOLIC EXTRACTS OF *Solanum nigrum* (Subsp *villosum)* IN HIGH FAT FED SPRAGUE DAWLEY RATS

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DECLARATION

I declare that the work presented in this thesis is my original work and has not been presented for the award of a degree in any other university or any other award.

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DEDICATION

I dedicate this thesis to my Parents, my siblings, my nieces and nephews

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ACRONYMS AND ABBREVIATION

ALV - African Leafy Vegetables

ANOVA - Analysis of Variance

BMI - Body Mass Index

DMEM - Dulbecco's Modified Eagle's medium

HDL–C - High Density Lipoprotein cholesterol

LDL-C - Low Density Lipoprotein cholesterol

NP-Y - Neuro Peptide Y

OECD - Organization of Economic Co-operation and Development

PBS - Phosphate buffered saline

POMC - Pro- opiomelanocortin

PPARs - Peroxisome proliferator activated receptors

TC - Total cholesterol

TG - Triglyceride

VLDL - Very Low density lipoprotein

WAT - White Adipose Tissue

WHO - World Health Organization

ABSTRACT

Despite the rapid rise of obesity in Kenya, its treatment and management has remained a challenge due to its high side effects and unaffordability. Locally based remedies may offer cheaper, safer and more cost effective alternatives.

Materials and Methods

This study sought to evaluate the anti-obesity effects of *Solanum nigrum* (Subsp *villosum)* extracts in high fat fed Sprague dawley rats. The study utilized 35 Sprague dawley rats weighing between 160-180g. Experimental rats were divided into seven groups of 5 rats each; normal control group (fed with normal chow rat feed), a negative control group (fed with high fat diet), reference positive control group (orlistat 30mg/kgbw with high fat diet) and experimental groups (treated with Dichloromethane and methanolic leaf dose extracts of *Solanum nigrum* (Subsp *villosum)* at concentrations of 150mg/kgbw and 300mg/kg bw respectively plus high fat diet). The body weight and lengths (naso-anal length) were measured once per week for eleven weeks to determine the body mass index. At the end of the experiment, blood samples were obtained through cardiac puncture. They were centrifuged at 2400rpm for 10 minutes and the serum obtained was used for clinical chemistry. In addition, caecum samples were collected for microbiome analysis and molecular identification of *Solanum* done using internal transcribed spacer 2 (ITS2) and ribulose biphosphate carboxylase large (RBCL) marker. Statistical analysis was conducted using Student's t-test and one way anova.

Results: Leaf extracts of *Solanum nigrum* (Subsp *villosum)* at dose of 150mg/kgbw and 300mg/kgbw, showed significant decrease on body mass index (BMI) from week 5-11 compared to the negative control ($p<0.05$). However, low dose of dichloromethane (150mg/kgbw) and high dose of methanol extracts (300mg/kgbw) showed better decrease in BMI than all the other treatment groups. There was no significant difference on total cholesterol among the treatment groups compared to the negative control ($p>0.05$) however, a significant difference was seen on triglycerides, low density lipoprotein- cholesterol and high density lipoprotein- cholesterol among the treatment groups compared to the negative control. There was a significance difference on adipose tissue weights and liver weight on rats administered with high fat diet compared to other treatments ($p<0.05$). The rats on high fat diet (negative control) indicated a significant increase on alanine aminotransferase, gamma- glutamyl transferase and alkaline phosphatase compared to the other treatment groups. Decrease on adipose tissue and liver weights on other treatments compared to the negative control was significant ($p<0.05$). These extracts showed presence of various phytochemical compounds. Microbiome analysis exhibited that all groups had varying amounts of bacteria phylum. However, Campylobacterota had a significant increase on the DCM and methanolic treatment groups as well the positive control compared to negative and normal control groups. On the other hand, the different sequences of plant markers (ITS2 and rbcl) used to infer phylogeny indicated that the specie with antiobesity effects (*Kat_001)* is *Solanum nigrum* (Subsp *villosum)* specie.

Conclusion: *Solanum nigrum* (Subsp *villosum)* reduces BMI, has hypolipidemic effect and possess anti-obesity phytochemicals with no adverse effects on liver function suggesting its safe use.

High fat diet and *Solanum nigrum* (Subsp *villosum)* extracts caused significant changes in the caecum microbial system of the high fat fed diet rats. These results were similar to those of positive control.

The agreement between rbcl and the combined set of rbcl and ITS2 sequences, together with morphological data strongly suggests *Kat_001* is *Solanum nigrum* (Subsp *villosum).* The study recommends consumption of *Solanum nigrum* (Subsp *villosum)* as a supplement used to prevent obesity.

CHAPTER ONE

INTRODUCTION

1.1 Obesity

One of the most common non-communicable diseases in the world is obesity, which is brought on by an imbalance between the amount of energy used and the amount consumed. It is reported to have affected 60% of adults and nearly one in three children (27% of girls and 29% of boys) (Organization, 2022). Its primary characteristics include high blood lipid levels and excessive fat mass, while extra calories from the diet are deposited as triacylglycerides (TAG) in the white adipose tissue (WAT) (Jang & Choung, 2013; Stadler & Marsche, 2020). The increased body fat mass has led to a higher body mass index of (BMI $\geq$ 30 kg/m^2) which is considered as obese by the world health organization (Organization, 2020). Fat mass increase occurs through two processes these include adipocyte hyperplasia or adipocyte hypertrophy (Shao *et al*., 2018). Adipocyte hypertrophy which is characterized by enlargement of fat cells due to infiltration of M1 macrophage, decreased vessel development and massive fibrosis leads to morbid obesity (Farias *et al*., 2019). Hence leading to chronic inflammation and white adipose tissue (WAT) dysfunction, this dysfunction is mainly associated with obesity associated medical comorbidities (Ni *et al*., 2020).

Current studies indicate that gut microbiome plays a part in the growth of gastro intestinal diseases, metabolic pathologies such as insulin resistance, obesity and neurological disorders such as Alzheimer, Parkinson and autism (Ghaisas *et al*., 2016 ; Cryan *et al*., 2020). The gut consists of trillion microorganisms which originates at birth into early childhood (Deschasaux *et al*., 2018). Early childhood microbiome plays a vital

part in the nervous and immune system development and have also showed to affect motor control and anxiety in adulthood (Noble *et al*., 2017; Warner, 2019). The gastrointestinal tract plays major role in regulation of energy intake and appetite for instance several studies indicate that obese persons have less sensitivity to the gastrointestinal effects of nutrients, especially in excess calorie intake (Monteiro & Batterham, 2017). Several research studies also report the association between gut microbiota and obesity related diseases. A study by (Zhang *et al*., 2015) indicated that *Enterobacter cloacae* B29 isolated from the gut is associated with both obesity and insulin resistance. This indicates that gut microbiota contributes highly to metabolic disease and maybe a drug target for treatment (Zhang *et al*., 2015). However, low calorie intake, prebiotic rich diets and healthy intestinal microbiome reduces obesity incidence by reducing production of short chain fatty acid (Murugesan *et al*., 2018). Further studies also show that pre- biotic rich diets may decrease factors associated with metabolic syndrome, obesity, triggering of the immune system (Jakobsdottir *et al*., 2014), reduction of excess circulating glucose, reduction of blood cholesterol levels, and improvement of insulin sensitivity (Johnson *et al*., 2013).

Obesity is also as a result of hormonal imbalance such as increase of ghrelin hormone in the stomach and reduction of the leptin hormone in the adipose tissue. (Cui *et al*., 2017). Research shows treatment with leptin slows down the hyperphagic effect, reduces body fat mass and as well increases energy expenditure in obese persons (Schwartz *et al*., 2017). Gut hormones are regulated by gut microbiota which mostly influence production of short chain fatty acids such as acetate, butyrate and propionate (Lin *et al*., 2012). They are known as signaling molecules for certain receptors in the gut for example, free fatty

acid receptor 2 and 3, (FFAR2 and FFAR3), a G- coupled receptor free fatty acid have been recognized as endogenous receptor for the short chain fatty acid (Lin *et al.*, 2012). Previous studies show that propionate inhibits food intake in human by regulating peptide hormones secreted by the enteroendocrine cells. These hormones include; Glucagon like peptide 1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) which are key modulators of glucose metabolism (Mosavat *et al*., 2020). Obesity has also been reported as a chronic medical condition risen as a result of multiple genetic factors, for example genes such as fat mass and obesity associated (FTO) genes play a crucial part in the contribution to obesity (Bjørnland *et al*., 2017). Few people have monogenic form of obesity, for example, monogenic mutations on the leptin hormone, leptin receptor and on the melanocortin-4 receptor lead to about 10% of obesity cases (Kohlsdorf *et al*., 2018). However, research shows that almost 200 single gene mutations also cause obesity (Albuquerque *et al*., 2015). Syndromic forms of obesity are also associated with genetic disorders leading to change of phenotype these examples include; mental retardation, Wilms tumor, Prader willi syndrome, Cohen syndrome, and genitourinary anomalies among others (Thaker, 2017). Other studies indicated that epigenetic factors such as changes in noncoding microRNAs, DNA methylation and microRNA expression contribute to obesity, since epigenetics are likely to change through lifestyle modification such as diet and physical inactivity (Izquierdo & Crujeiras, 2019).

Obesity is not independent in humans, it's mainly associated with various risks such as type 2 diabetes mellitus, hypertension, dyslipidemia, cancers, retinopathy, steatosis hepatitis and this combination is known as metabolic syndrome (Weihrauch-Blüher *et al*., 2019). It is known as metabolic syndrome since it's mainly as a result of dietary

imbalance rather than genetically programmed disease (Aguilera *et al*., 2013). Additionally, fatty liver disease that is not caused by alcohol is more likely to develop in people who are obese (Suzuki *et al*., 2012).

Prevalence of obesity has continuously increased and therefore led to high mortality rates in the globe. Around 500 million persons were obese and 1.5 billion were overweight in 2008 (Nguyen & Lau, 2012). In addition, in 2010, 43 million children (below 5 years) were overweight (Organization, 2017). According to Popkin, his study indicated that in the year 2012 approximately 300 million adults were obese while 1 billion adults were overweight globally (Popkin *et al*., 2012). Moreover, another study indicated that 2.8 million people of global population die due to overweight and obesity per year while about 35.8 million are left with disability (Steyn & Mchiza, 2014). In 2014, World Health Organization reported 13% of global adult population were obese (Organization, 2015a) and in 2016, over 1.9 billion adults over the age of 18 had obesity and/or overweight, with 650 million of them being obese (Organization, 2020). WHO also indicated that among 39% of adults aged above 18 years, 39% were men while 40% were women. The report similarly indicated that 13% of global obese population in 2016; 11% were men while 15% were women. The global obese population nearly tripled between 1975 and 2016 while in 2019, there were 38.2 million obese or overweight children under the age of five (Organization, 2021).

Obesity was previously considered as a high-income country problem; however, both middle-income and low-income countries, especially those with metropolitan contexts, have seen an increase. Overweight and obesity affect 27% and 8%, respectively, of

persons in Africa over the age of 20 (Steyn & Mchiza, 2014). According to WHO, children considered overweight under 5 years of age have nearly increased by 24% percent since year 2000 (Organization, 2021). In 2016, 340 million children and adolescent between 5-19 years of age were obese or overweight, this report showed that there was an increase of prevalence of obesity or overweight from 4% in 1975 to over 18% in 2016. In 2016, there was also an increase in the percentage of overweight boys and girls, with 18% and 19% of both being overweight (Organization, 2021). Recent studies indicate that obesity and overweight prevalence has increased in Sub-Saharan Africa, including Kenya at an average rate of 5% per annum (Ziraba *et al*., 2009). In Kenya 37.5 % of women and 17.5% of men were either obese or overweight while 4% of the children under 5 years were obese or overweight (Kihiu & Amuakwa-Mensah, 2021). A study done by (Mkuu *et al*., 2018) indicated that 1 of 3 Kenyan women are either overweight or obese.

There are lots of factors that lead to obesity and these include the nutrition transition which is mainly driven by demand and supply factors (Jayawardena *et al*., 2017). For example, urbanization, improved education and income growth has led to the increase in food demand and less physical activity hence increasing sedentary nature (Sturm & An, 2014). Other factors also include globalization which has contributed much to the preference shifts towards westernized diets (Costa-Font & Mas, 2016; Monteiro & Batterham, 2017). On the other hand, food systems have now become more international and the influence of the multinationals is growing rapidly (Monteiro *et al*., 2013). Retail

sectors are now modernizing and consolidating hence many people have ended up consuming energy dense processed foods (Garnett & Wilkes, 2014).

Other factors leading to obesity include sedentary lifestyle for example, excessive sedentary behavior such as physical inactivity and white collar jobs, short sleep duration, psychiatric issues among others (Kang *et al*., 2020). According to WHO, 60% of the global population are inadequate of exercise due to prevalence of labor saving technologies in production industries as well as at home and increase of mechanized transportation. WHO also indicated that television viewing in adults and children has a great connection with high risks of obesity (Hills *et al*., 2011). Psychiatric patients are also highly likely to suffer from obesity unlike the persons without psychiatric illnesses. For example psychiatric persons may suffer from binge eating and night syndrome (Rankin *et al*., 2016). There is however an urgent need for safe and efficient therapeutics for obesity, since the present status of such drugs is still unsatisfactory (Hong *et al*., 2015).

Obesity therapies include use of drugs that reduce fat absorption such as orlistat (Liu *et al.*, 2020*)*. Orlistat drug inhibits the pancreatic lipase thus reducing the intestinal fat absorption. Other drugs include appetite suppressant or anorectic drugs they include drugs such as sibutramine among others (Yun, 2010). However, research indicates that these drugs have harzadous side effects. For example, both (orlistat and sibutramine) side effects include dry mouth, headache, blood pressure, constipation and insomnia. For this reason, current approaches majors on the natural sources that have been said to have anti-obese effects (Nazish & Ansari, 2018). Although the use of natural sources as an anti-obese agent have not been fully explored, a few edible plants with medicinal value are

used as supplements for management of weight in many countries (Matic *et al*., 2018). Studies reports numerous bioactive compounds such as alkaloids, steroids, tannins, saponins extracted from natural sources are potentially useful in obesity management with minimal side effects (Konstantinidi & Koutelidakis, 2019). Thus, it might be a perfect alternative strategy for developing safe and effective antiobesity medicine in future.

This study shall utilize a specie from solanaceae family known as *Solanum nigrum* which is a complex of many species such as *S. scarbum, S. americanum, S. nigrum, S. physalifolium and S. villosum* (Hameed et *al*., 2017). It is well known as Managu in Kenya and has been used all over the world for a variety of treatments, including the control of human bacterial infections, digestive problems, diabetes, and high blood pressure, among others (Matasyoh & Bosire, 2016). Folklore in Kenya suggests that the plant managu has anti-obesity properties, although this claim has not been scientifically validated. Therefore, the focus of this study was on evaluating the value of the specie's ethnomedical anti-obesity properties in rats on a high-fat diet.

1.2 Problem Statement

Obesity epidemiology has increased in both industrialized nations as well as the developing countries globally (Yun, 2010). It is ranked as the fifth most outstanding cause of mortality around the globe and is projected to be among the top three cause of death by 2030 (Organization, 2021). In Africa, children under 5 years of age with cases of overweight and obesity nearly doubled from year 1990 to 2014 from 5.4 million to 10.3 million (Organization, 2015b). In accordance to World Health Organization, the percent of overweight in children under 5 years of age has nearly increased to 24% from year 2000 to 2019 (Organization, 2021).

Recent studies show that obesity incidence is due to disproportion between food expended and food consumed (Blüher, 2019). This has resulted due to consumption of palatable foods, sedentary lifestyle, lack of exercise and a decrease in vegetable consumption (Aparicio *et al*., 2016). There are various risks associated with obesity which include; hyperlipidemia, insomnia, sleeps apnea, diabetes mellitus, coronary diseases, hypertension among others (Medic *et al*., 2017). Gut microbiome dysbiosis which is as a result of change in di*et al*so lead to metabolic diseases such as obesity among others (Belizário *et al*., 2018). Although pharmacological approach to control the prevalence of obesity has been investigated, only a few of the synthetic drugs have been permitted for clinical use, owing the hazardous side effects associated with them. For example, conventional antiobesity drugs such as orlistat are reported to have severe side effects which include; constipation, blood pressure, dry mouth, headache and insomnia (Kim *et al*., 2020).

1.3 Justification

Recent approaches are focusing on screening of natural agents that have been reported to have anti-obesity effects with minimal side effects and potentially act as a substitute for synthetic medications (Rodríguez-Pérez *et al*., 2019). Current research focus is on phytochemical compounds capable of reducing gut dysbiosis, as a potential drug target for metabolic diseases (Sheflin *et al*., 2017).

This study will scientifically assess the ethno-medicinal significance of *Solanum nigrum* (Subp *villosum)* a complex of many species, on the diversity of gut microbiome and its anti-obesity effects on high fat fed rats. Although, it has a widespread folklore claim to prevent obesity, there is no documentation of their claimed effects that has been scientifically validated.

1.4 Research Question

i. Do Dichloromethane - methanolic leaf extract of *Solanum nigrum* (Subsp *villosum*) have any effect on body mass index, and food consumption in high fat fed rats?

ii. Do DCM - methanolic leaf extract of *Solanum nigrum* (Subsp *villosum*) have any effect on glucose, lipid profiles (HDL-C, LDL-c, TG, VLDL-C and TC) and hepatic enzymes (alanine amino transferase, Gamma- glutamyl transferase and alkaline phosphatase) in high fat fed rats.

iii. Do DCM- methanolic leaf extract have effects on the diversity of caecal microbiome of high fat fed rats

iv. Which specie of *Solanum* genus showed anti- obesity effects in high fat fed rats

1.5 Objectives

1.5.1 General Objective

The general objective of the study is to determine the anti- obesity and ceacal microbiome effects of DCM - methanolic leaf extracts of *Solanum nigrum* (Subsp *villosum)* in high fat fed rats.

1.5.2 Specific Objectives

i. To determine effects of DCM - methanolic leaf extracts of *Solanum nigrum* (Subsp *villosum*) on body mass index and food consumption in high fat fed rats.
ii. To examine the effects of DCM - methanolic leaf extracts of *Solanum nigrum* (Subsp *villosum*) on glucose, lipid profiles (TG, LDL, HDL, TC and VLDL) and Gamma- glutamyl transferase, alkaline phosphatase and alanine amino transferase in high fat fed rats
iii. To assess the diversity composition of caecal microbiome on high fat fed diet rats
iv. To explore the *Solanum nigrum* sub specie used as an anti- obese agent on high fat fed rats

CHAPTER TWO:

LITERATURE REVIEW

2.1 Obesity

Obesity comes from a latin word obesitas meaning fat, plump or stout. It is a metabolic disorder, which results to an increase in adipose tissue due to an imbalance between energy consumed and energy expended (Mohamed *et al*., 2014a). According to several studies, obese people incur health care cost of above 25% than healthy people (Withrow & Alter, 2011). Obesity can be iatrogenic due to treatments such as antiepileptic, antidepresent, insulin, steroids and or due to other diseases such as cushing syndrome, hypothalamic defects and hypothyroidism (Kuete, 2017; Mohamed *et al*., 2014a). Obesity is frequently associated with various risks such as steatosis hepatis, sleep apnea, type 2 diabetes, cardiovascular diseases dyslipidemia, dementia and cancers of the pancreas and urinary bladder (Avgerinos *et al*., 2019; Blüher, 2013). This metabolic disease could also result from a multifaceted interaction of behavioral, environmental and genetic factors (metabolism) that correlate with the social, lifestyle (physical inactivity) and economic status (Mansur *et al*., 2015). Positive energy imbalance is mostly related to change in diet, low metabolic rate and sedentary lifestyle among others. Current approaches are focusing on lifestyle and diet modification for example, restriction of calorie intake and increased bodily exercise to reduce prevalence of obesity (Câmara *et al*., 2017). Several scientists have shown the possible use of natural agents to counter obesity. This use of natural products may result in a synergistic manner thus increasing its bioavailabilty and action on the molecular markers, hence offers advantage over the synthetic drugs (Mohamed *et al*., 2014a). The anti- obese effects of this compounds are associated with

the down regulation of several pathways such as decreasing lipogenesis, increasing lipolysis, decreasing differentiation and proliferation of preadipocytes, energy intake, expenditure and lipid absorption (Huang *et al*., 2014).

2.1.1 Epidemiology of Obesity

The ratio among obese people varies among different ethnicities, countries, sex and is dependent on behavioral and environmental changes brought by urbanization, modernization and economic development (Williams & Periasamy, 2020). The variation in the incidence of obesity could be attributed to the sedentary lifestyle, lack of physical inactivity, consumption of easily available palatable foods, sex, heredity and age (Pan *et al*., 2021). Previous data on obesity and overweight from the organization of economic co-operation and development (OECD) indicated that at least 13 member countries had half of the adult population obese or overweight (Devaux & Sassi, 2013). These countries included; Spain, United Kingdom, United States, Australia among others. The report also indicated that non- OECD countries such as South Africa, China, Brazil among others had rates lower than OECD countries but were increasing at the same rate (Devaux & Sassi, 2013; Hruby & Hu, 2015). World Health Organization indicated that international obesity prevalence nearly tripled since 1975 to 2016 from 4% to 18%, these rises occurred in both boys and girls at 18% and 19% respectively (Organization, 2021). In 2014, 41million global population of children under 5 years of age were either obese or overweight (Organization, 2015b). Global prevalence of obesity in 2016 indicated that among 1.9 billion adults who were overweight, 650 million were obese. The study also indicated that 340 million children and adolescent between 5-19 years were either obese or overweight in 2016 (Organization, 2020). An overall study indicated that 13% of

global population were obese in 2016 among them included 11% men and 15% women. On the other hand, in 2019, 38.2 million people under 5 years were either obese (Organization, 2020) or overweight. In 2020, 39 million children below 5 yrs age were either obese or overweight (Organization, 2020). Obesity is a public health concern not only in the industrialized countries but has also trickled down to middle- and low-income countries particularly in metropolitan settings (Organization, 2020). In Africa, children under 5 years of age with cases of obesity and overweight nearly doubled from year 1990 to 2014 from 5.4 million to 10.3 million. Of these, 48% lived in Asia and 25% in Africa (Organization, 2015b). In accordance to World Health Organization, the percent of overweight in children under 5 years of age has nearly increased to 24% from year 2000 to 2019 (Organization, 2015b). Overweight and obese are linked to more cases of mortality than underweight, while around the globe more people are obese than overweight. Linear time trend forecasts predicted that 51% population might be obese and nearly 1 in 2 adults will be obese in 2030 (Finkelstein *et al.*, 2012; (Keaver *et al*., 2013).

2.1.2 Etiology/Pathophysiology/chronobiology of obesity

Life is rhythmic occurrence whereby our internal body system entirely depends on yet we rarely pay attention to it (Garaulet *et al*., 2010). These rhythmic oscillations originated from the word *circa diem* and is known as circadian rhythm. The principal function of this internal circadian rhythms in organisms is to anticipate and predict conservational changes while trying to acclimatize to their physiological and behavioral functions (Gómez-Abellán *et al.*, 2012). In humans, habits such as disruption of everyday sleep wake rhythm, reduction in sleep, introduction to bright light at night and increase in

consumption of junk food induces perception loss to the external and internal rhythms (Aulinas, 2019). Circadian rhythm is known to control both hormones and cardiovascular function involved in metabolism such as cortisol, leptin, glucagon, insulin, growth hormones among others (Kim *et al*., 2015). Hence, studies indicate that disruption of circadian rhythm maybe a cause of various metabolic syndromes and obesity contraindications such as hypertension, dyslipidemia, glucose intolerance, type 2 diabetes mellitus, cardiac diseases, endothelial dysfunction among others (Farhud & Aryan, 2018). Daily rhythms that are involved in satiety, hunger signals and meal times are based not only on what should be eaten but also when to be eaten and degree of obesity (Garaulet *et al.*, 2010).

The central pacemaker which is located at the suprachiasmatic nucleus (SCN) of the hypothalamus is the key component of the circadian system (Honma, 2018). However, in natural environment settings the suprachiasmatic nucleus is readjusted by various periodic inputs such as food intake/fast, exercise and light /darkness. This system is controlled by the central pacemaker and the peripheral clocks. The central pacemaker is located at the hypothalamic suprachiasmatic nucleus, where it generates a 24 hour cycle in both behavioral and physiological variables by controlling the expression of the circadian rhythm (Ono *et al*., 2018). In addition, expression of circadian rhythm is controlled by a number of genes known as clock genes, which encode various proteins hence generating a self- regulatory mechanism using positive- negative transcription feedback loops (Honma, 2018). The positive elements involved in the feedback have a helix- loop –helix structure and they include circadian locomotor output cycles kaput and brain and muscle ARNT-like protein containing Per-ARNT-Sim domains which help

with protein-protein interactions. An alternative to CLOCK includes a homolog Neuronal PAS domain protein 2 (NPAS2) which effectively compensates for CLOCK absenteeism. A heterodimer is then formed between the two positive elements CLOCK/BMAL1 which attaches to the promoter of various clock genes such as period 1, 2, 3 and cryptome 1 and 2 and retinoid – related orphan receptor -ὰ (Ror ὰ) among genes that are controlled by CLOCK (Doruk *et al*., 2020).

Lipid metabolism is controlled by Clock proteins such as BMAL1 and other CCG proteins (PPAR ὰ and REV- ERB ὰ. Clock and BMAL1 are also a part of the control of glucose levels while PER2 and CLOCK are appetite regulators (Yao *et al*., 2020).

Few studies on circadian biology of the adipose tissue have been done on rodents and finding show that rodents with clock gene mutation are characterized as obese without circadian biology rhythm while *Bmal* deficient mice were found to have high amount of adipose tissue. The study also indicated deficiencies and mutation of adipokine genes, leptin and melanocortin receptors which are associated with obesity and phenotypes resembling metabolic syndromes due to a defective circadian rhythm (Garaulet *et al*., 2010).

2.1.3 Endocrinology of Obesity

Hormones such as leptin, adiponectin and acylation stimulating protein (ASP) have a crucial role in regulation of carbohydrate and lipids (Harwood, 2012). The leptin hormone main biological role is to reduce the energy available rather than prevent obesity by providing signals to the brain on the quantity of fat stored (Cui *et al*., 2017). Body adiposity increases in people with leptin gene mutations since they have very low circulating leptin levels. However, leptin treatment significantly decreases body

adiposity, improves metabolic abnormalities (hyperlipidemia and insulin resistance) in patients with fairly leptin deficiency (Wu *et al*., 2019). Leptin production is enhanced by insulin, glucocorticoids and estrogen while on the other hand it's reduced by β-adrenergic agonists (Qaid & Abdelrahman, 2016). Moreover, dietary fat and fructose, does not increase insulin secretion, hence, leads to decrease in leptin hormone (Bhaswant *et al*., 2015).

In obesity state, the leptin hormone produced by the adipose tissue inhibits Neuropeptide Y secretion at the hypothalamus thus leading to a decrease in appetite, increase in energy expenditure as well as stimulates breakdown of stored fats (Zhang *et al*., 2014). Insulin sensitizing hormone such as adiponectin are also derived from the adipocytes and are secreted into the blood (Lee & Shao, 2014).

A few studies report that serotonin hormone role in management of obesity is to reduce food consumption and increase the energy expended. Serotonin enhances melanocortin 4 receptors (MC4) which regulates food ingestion in the hypothalamus (Sargent & Henderson, 2011). Previous studies demonstrate inverse relationship between food intake and serotonin hormone. The three serotonin receptors associated with food intake control include 5-hydroxytryptamine (5- HT1B, 5-HT6 and receptor 5HT2C) (Voigt & Fink, 2015).

2.1.4 Genetics of Obesity

Obesity disease has highly escalated in the past few decades humoral and behavioral factors are considered as causes of obesity (Upadhyay *et al*., 2018). However, in spite of people's sedentary lifestyle, lack of physical exercise, increased food intake and less energy expended not all individuals are obese due to this factor, change in genetic

mechanisms has led to obesity. There are several different genes that have been linked to obesity; some act alone, while others work in combination. The most studied fat gene include the fat mass and obesity associated (FTO) gene, this gene is said to work well on its own, however, it can be modified by people's lifestyle (Albuquerque *et al*., 2017). Obesity inherited in a Mendelian pattern is known as monogenic obesity and it results from large or small chromosomal deletions or single gene defects. However, only 200 single genes mutations have been recorded to cause obesity (Loos & Yeo, 2022). Leptin receptor mutations or leptin and melanocortin-4-receptor mutations are significant monogenic mutations that account for less than 10% of cases of obesity (Wasim *et al*., 2016). The other category is known as polygenic obesity or common obesity and is as a result of various polymorphism. The variants causing this type of obesity include BDNF, CNR1, MCAR, CSKI and PPARG (Loos & Yeo, 2022).

2.1.5 Obesity classification

There are two types of obesity, these include; central and peripheral obesity (Huang *et al*., 2020). Obesity is classified based on abdominal fat in the body (Gómez-Ambrosi *et al*., 2012). Fat distribution around the upper body and trunk is known as central obesity while even distribution of fat in the body which predominantly occur in women is known as peripheral obesity (Booth *et al*., 2014). Central obesity entails fat distribution in areas such as chest, abdomen, shoulder and nape of the neck. This leads to an apple shape hence referred to android obesity and is common in males. Peripheral obesity is also known as gynoid obesity and is commonly found in women whereby it becomes difficult to differentiate a woman from a man. Fat distribution is mainly on the thighs, hips and bottom, leading to a pear shape (Neri *et al*., 2020 ; Shin & Saeidi, 2021). Previous studies

indicate that during adulthood fat distribution in women is evenly distributed however as they bear children, age and nearing menopause their fat distribution changes to android. Body mass index and waist hip ratio are the anthropometric measurement for obesity. According to WHO BMI above 30 and waist hip ratio of men and women of 0.9 and 0.85 respectively indicate obesity (Gazarova *et al*., 2019). Android obesity is mostly associated with several metabolic diseases such as diabetes mellitus, hyperinsulinemia, hyperlipidemia, insulin resistance, cardiovascular diseases among others. However, gynoid obesity shields against cardiovascular diseases (Scholz & Hanefeld, 2016).

2.2 Management of Obesity

Anti-obesity drugs are classified based on their primary function and are divided into four groups as described below:

2.2.1 Fat Absorption Inhibitor Drugs

Drugs such as orlistat fall under this category, they reduce weight by inhibiting lipases in the small intestines, stomach's mucous membrane and pancreas (Xuan *et al*., 2017). Hence, preventing hydrolysis of triglycerides into absorbable monoacylglycerols and free fatty acids and their absorption in the intestine (Kim *et al*., 2020). Previous studies show that they reduce energy intake by gastrointestinal mechanism of action and not appetite (Son & Kim, 2020).

2.2.2 Stimulators of Thermogenesis

These drugs increase thermogenesis without physical activity. Adaptive thermogenesis confers to overfeeding (diet-induced thermogenesis) and the ability to adapt cold (Bastías-Pérez *et al*., 2020). A notable influence on adaptive thermogenesis is exerted by

thyroid hormone produced from sympathetic neurons; examples include the medication Ligratude (Oliveira *et al*., 2021).

2.2.3 Appetite Suppressing Drugs

These drugs include noradrenergic, serotoninergic and mixed noradrenergic serotoninergic agents such as Brupropion, phentermine, phendimetrazine, bezphetamine, dexfenfluramine and sibutramine (Shettar *et al*., 2017). Previous studies report that these drugs reduce food consumption by blocking the reuptake of monoamines, increases feeling of satiety and acts on the central nervous system (de Matos Feijó *et al*., 2011). Appetite suppressing drugs also inhibit norepinephrine and serotonin re-uptake in the Central nervous system, while also suppress appetite by reducing hunger perception (Ueno & Nakazato, 2016). For example, a dopamine and norepinephrine inhibitor such as bupropion activates neuropeptide Pro-opiomelanocortin (POMC) hence decreasing appetite when highly concentrated in the hypothalamus and supplements dopamine activation which is lower in obesity patients (Chen, 2016). Naltrexone drug inhibits appetite enhancing effects of β –endorphin caused by cannabinoid -1- receptor activation. Drugs such as naltrexone and bupropion have synergistic effects when combined and hence lead to appetite suppression (Subramaniapillai & McIntyre, 2017; Wilding, 2017). Some drugs such as Topiramate an epilepsy drug treats obesity through exerting weight reduction by increasing energy expenditure, satiety and reducing calorie intake while phentermine reduces appetite by increasing epinephrine secretion in the hypothalamus. Fenfluramine and dexfenfluramine act by releasing serotonin (5-HT) which reduces food intake especially fat intake and increases satiety (Georgescu *et al*., 2021; Paccosi *et al*., 2020; Tak & Lee, 2021). In addition, Fluoxetine is an inhibitor for serotonin re-uptake

hence reducing food intake (Farhan *et al*., 2013). However, though some of these drugs have been approved for short term use, they increase insomnia, anxiety and depression (Son & Kim, 2020).

2.2.4 Stimulators of Fat Mobilization

Drugs that stimulate fat mobilization act peripherally by reducing triglyceride synthesis and fat mass without decreased food intake or physical exercise. This occurs by stimulating formation of brown adipose tissue from inter-conversion of white adipocytes or pre-adipocytes (Chatzigeorgiou *et al*., 2014).

2.2.5 Future anti-obesity drugs

Glucagon like peptide 1 is a hormone released to stimulate secretion of insulin in the gut due to elevated glucose (Nauck *et al*., 2011). Drugs such as gliptins stimulates break down of incretins by dipeptidyl peptidase IV enzyme (Coppolino *et al*., 2018). Other drugs include liraglutide and exanatide which mimic glucose like peptide 1 by reducing weight loss on type 2 diabetic patients through glycaemic control (Lyseng-Williamson, 2019).

2.3 Limitations of Conventional Drugs

Most conventional drugs are said to be expensive and also have been reported to have severe side effects, for example they increase blood pressure, dry mouth, tachycardia, insomnia, constipation, headache incontinence, flatulence with discharge and steatorrhoea (Daneschvar *et al*., 2016).

2.4 Alternative complementary medicine of obesity

Various herbal plants are used in the management of obesity (Hasani-Ranjbar *et al*., 2013). For example, a study done on obese mice showed that ethanolic leaf extract of *pomegranate* at a dose extract of 50 mg/kg and dose extract of 100 mg/kgbw exhibited a significant decrease in the body weight in comparison with the control group, which was only fed on high fat feed (Anusuya *et al.*, 2010). Ethanolic extract of cowpea seeds also showed a decrease in serum total cholesterol concentration, LDL-cholesterol, triacylglycerol and also glucose in rats when compared to high fat fed rats fed (Weththasinghe *et al*., 2014).

A study done by Bais *et al*. 2014 to evaluate the antiobesity activity of methanolic extract of *Moringa oleifera* leaves (MEMOL) in high fat fed rats, reported that dose extracts of Memol led to a considerable decrease in low density lipoprotein, total cholesterol, body weight and triglyceride. In addition, treatment with MEMOL extracts demonstrated a hepatoprotective effect, indicating decreased levels of serum glutamic pyruvic transaminase (SGPT) (Bais *et al*., 2014). To evaluate the antiobesity and hypolipidemic effects of *Momordi cadioica* fruits extracts, a study showed a significant decrease in serum LDL-C, TG and TC at a dose extract of 500mg/kgbw (Safia & Krishna, 2013). *Cucucurbita pepo* dose extracts also demonstrated a significant decrease in LDL, TG and glucose in male diabetic rats (Asgary *et al*., 2011).

Among other natural products that have antiobesity effects include green tea whose presence of phytocompounds contributes to its effect (Xu *et al*., 2015). The compounds that were found present in the green tea include; Flavanols such as catechin (C), Catechin 3- gallate (CG), Epigallocatechin gallate (ECG) whose results indicated that they

suppressed lipid accumulation in the adipocyte cells, hence this study concluded that catechin and epigallocatechin-3-gallate (EGCG) inhibits differentiation and proliferation of 3T3- L1 adipocytes by inhibition of phosphor- ERK1 and phosphor- ERK2 pathway (Jiang *et al*., 2019).

A study done on hypolipidemic and antiobesity effects of *persimmon* leaves on high fat fed rats indicated that tannins or tannins with a gallate group contributed to anti-lipidemic effects on the hypercholesterelemic rats. This study also indicated that flavonoids of citrus peel extract contain the hypocholesterolemic effects since it demonstrated reduction of serum low density lipoprotein in rabbits (Zou *et al*., 2014).

The effects of Polyphenols or flavonoids on lipid profiles were relevant to cardiovascular diseases, the study indicated reduction of low density lipoprotein- cholesterol, triglycerides, total cholesterol and serum leptin on high fat fed rats (El-Tantawy, 2015). In addition, a study on *Nelumbo nucifera* leaves indicated that alkaloids present induced apoptosis in 3T3-L1 preadipocytes while the flavonoids had anti- tumor activities and improved lipid metabolism in high fat diet treatment. This study also reported that the extract was responsible for anti-obesity and hypolipidemic activity (Sharma *et al*., 2015).

2.5 Solanaceae

Solanaceae family comprises of 93 genera, among them is *Solanum nigrum* (Subsp *villosum)* specie. This genus is widely distributed in the world for example countries such as Brazil have 260 species with 127 species endemic (Barros *et al*., 2018). *Solanum nigrum* (Subsp *villosum) L* is one of the most abundant specie among the *Solanaceae* family and has approximately 2000-3000 species (Mehmood *et al*., 2020). The genus has taxonomic challenges due to the high number of published names and its diversity. These

species include; *Solanum tuberosum* L (Potato), *Solanum lycopersicum* L (tomato), eggplant (*Solanum melongena* L), *Solanum scarbum*, Mill, European *Solanum nigrum (* Subsp *villosum)* Mill, *Solanum Americanum* Mill (American black night shade) as well as ornamental plants like *Schizanthus*, *Petunia*, *Lycium* spp (kraal honey thorn) among others (Ronoh *et al*., 2018). Recent approaches have focused on molecular identification of the genus. This genus varies from diploid to hexaploidy due to phenotypic plasticity, polyploidy, specific intra and inter specific hybridization (Gallego-Tévar *et al*., 2018; Särkinen *et al*., 2015).

2.5.1 Origin of *Solanum nigrum.* L species

European black night shade was known for its medicinal purpose by both greeks and romans during the ancient times (Pandey *et al*., 2016). In the 1st century AD the plant was known a *cucubalus, strychnon* or *strumus* and was recommendend against wounds, lumbago and stings (Särkinen *et al*., 2018a). Later in the Middle Ages the European treated *S. nigrum*. L as *Solanum Hortense* and considered it as unique to plants such as *Solanum somniferum* (*Withania somnifera*.L), *Solanum Halicacabum* (*Alkekengi officinarum Moench*) or *Solanum furiosum* common names that were used by Germans and French (Särkinen *et al*., 2018b). The name *nigrum* originated from the berries since scientists from Europe considered the diversity of fruit color as same plant (Edmonds & Chweya, 1997). In 1544, Mathioli described the berries as (fructu rotundo, uiridi, qui post maturitatem nigricat, aut fuluefcit) meaning the fruit was green at early stages then they turned to yellow-brown or black at maturity (Särkinen *et al*., 2015). In 1583, Dodens described the berries more clearly (per initia virentes, maturae veròe, aut nigricantes, aut rubentes, aut luteo coloris) which meant that berries are normally green during the early

stages then they turn to mature red, yellow or black when they are fully grown (Särkinen *et al*., 2018b). He described the plant as *Solanum nigrum* despite the differences in fruit color. In 1753, Linnaeus described *S. nigrum* as different taxa such as *S. villosum* (*S.nigrum var. villosum*), *S.nigrum* (*as var. vulgare*), *S.americanum* (*S.nigrum var patulum*), *S.scarbum* (*S.nigrum var guineenense*). In 1768, Miller renamed *Solanum nigrum* species as follows (*S. scarbum* Mill), *S.villosum* Mill, *S.rubrum* Mill, *S.luteum* Mill, *S.americanum* Mill (Gilmour, 1955).

John Gerald in 1957, described *Solanum Hortense* as *Solanum nigrum* which he also called it garden night shade and claimed it as a medicinal plant. He suggested that it was not poisonous but safe for use (Vorontsova & Knapp, 2016).

2.5.1.1 *Solanum nigrum* (Black night shade)

Black nightshade, or *Solanum nigrum*, is a plant that may be found in practically all ethnic groups. It is referred to as managu by the Kenyan Kikuyu community. It is a perennial shrub that thrives in wooded environments and can reach lengths of 4 to 8 cm and heights of 40 to 130 cm (Matasyoh & Bosire, 2016). Numerous ethnic groups around the world include *Solanum nigrum* as a source of food, and it is also utilized as a medicinal plant. For instance, the flower's berries, roots, stems, and leaves are all utilized medicinally. Its main characteristics are purple –black berries and white flowers. However, in India, red berries characterize the plant (Nandhini *et al*., 2014).

Few studies report that *Solanum nigrum* show ability to biosynthesize glycosylated alkaloids, flavonoids, steroids that are of therapeutic value. It presents various pharmacological activities like treatment of measles, cardiac pain, chronic fever, arthritis,

has anti-diarrheal, antibacterial and antipyretic effects among others (Jagatheeswari *et al*., 2011; (Jain *et al*., 2011; Muthuvel *et al*., 2020).

Figure 1. *Solanum nigrum* (Subsp *villosum)* (Limuru, Kiambu county)

2.5.1.2 Economical use of *Solanum nigrum* (Subsp *villosum)*

In Africa the most preferred vegetable includes *Solanum nigrum* (Subsp *villosum)* due to its accessibility and availability among the communities (Maseko *et al*., 2018). It grows as a weed in most regions and its nutritional value is associated with its therapeutic effects. Previous studies indicate that boiling of *S. nigrum* for more than 20 minutes lowers 75-89% of ascorbic acid content, while boiling the leaves using six times of water for 15minutes reduces the ascorbic acid content by 70% loss. The fruits contain 1.2% ash, minerals (phosphorous and iron) and vitamins, 5.6% carbohydrates, 0.6% fat and 2.5% proteins (Paciulli *et al*., 2017). *Solanum nigrum* (Subsp *villosum)* is known to have various therapeutic effects, for example previous studies indicate that the crude extract together with the isolated compounds have anti- proliferative activity on cancer cell lines.

Its antiproliferative activity was determined by examining its cytotoxicity and DNA fragmentation in treated cell (Nawaz *et al*., 2021). Other studies indicated that *Solanum nigrum* (Subsp *villosum)* extracts inhibits DNA damage against sarcoma cells in mice while the dried berries inhibited liver damage and compounds such as gallic acid, catechin, caffeic acid, epicatechin and narigenin possessed anti-oxidative activity (Guruvayoorappan *et al*., 2015). The glycoproteins extracted from *Solanum nigrum* (Subsp *villosum)* linn blocked antiapoptotic pathway of NF-kappaB, hence increasing production of nitric oxide by activation of caspase cascade's reaction thus leading to anticancer abilities (El-Atroush, 2020). A study on sprague dawley rats showed that fruit extracts have anti-ulcerogenic effects by inhibiting gastric lessions. *Solanum nigrum* (Subsp *villosum)* showed inhibitory effects on H+K+ ATPase hence increasing it cardioprotective properties, it also showed to have hypoglycemic effects when oral glucose test was evaluated on diabetic mice (Atanu *et al*., 2011; Mani *et al*., 2022).

A polysaccharide from *Solanum nigrum* (Subsp *villosum)* known as SN-ppF3 has previously indicated to have anti-tumor effect by modulating the immune response of a person hence its ability in fighting cancer (Razali *et al*., 2016). *Solanum nigrum (*Subsp *villosum)* is also known to have antipyretic and anti-inflammatory activities, this was shown by the decrease in edema induced in animal models (Jain *et al*., 2011). In addition, *Solanum nigrum* (Subsp *villosum)* whole plant was used to test its activity against bacterial strains and the study concluded that it can be used to treat infectious diseases caused by resistant microorganism (Vijilvani *et al*., 2020). On the other hand, fruit extracts of *Solanum nigrum* (Subsp *villosum)* significantly reduced defeacation frequency of diarrheal induced mice among others (Rani *et al*., 2017).

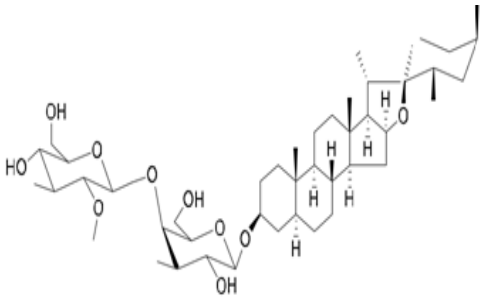
2.5.2 Secondary Metabolites of Solanaceae and their Biological properties

The compounds found in the genus *Solanum* include: 134 steroidal saponins, 63steroidal alkaloids, 72 flavonoids, 52 phenols, 66 sterols, 20 coumarins and coumestans, 13 pregnane glycosides, 128 terpenes, 31lignans, 312 types of alkaloids, 4 coumarinolignoids, 23 fattyacids and esters (Kaunda & Zhang, 2019).

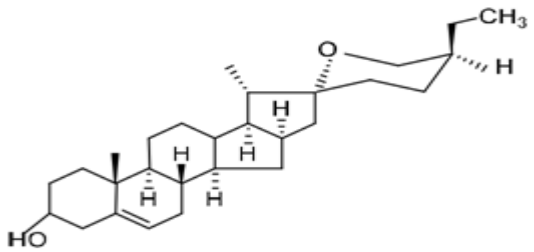
2.5.1.1 Steroidal Saponins

Steroidal saponins are the most outstanding bioactive compound in the *Solanum* (Kaunda & Zhang, 2019). Of these, *S. torvum* was the most studied whose results obtained 32 saponins including chlogenone (5α, 25S)- spirostan-3,6-dione, diosgenone, solanolactosides, torvosides from the fruits, leaves and the arterial part of the whole plant (Challal *et al*., 2014).

Spirostane saponins were also isolated from *S. chrysotrichum* leaves while lyconosides were reported to be present in the fruits of *S. lycocarpum* among others (Mohan *et al*., 2009). The *Solanum* saponins are reported to have various bioactivities such as anticancer, hepatoprotective, antimelanogenesis, antifungal, anti- inflammatory, anticonvulsant, cytotoxic and anti-viral properties (Banik *et al*., 2020).



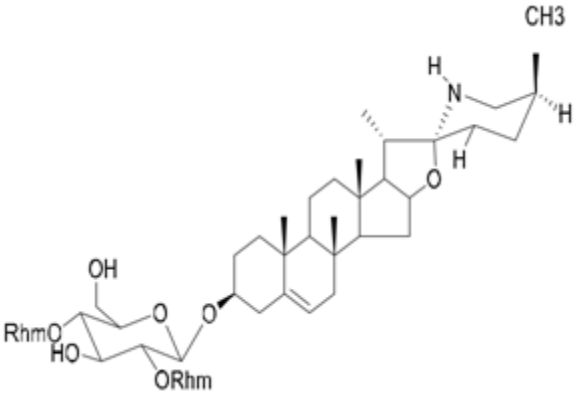
Inunigroside A (Ohno *et al*., 2012)



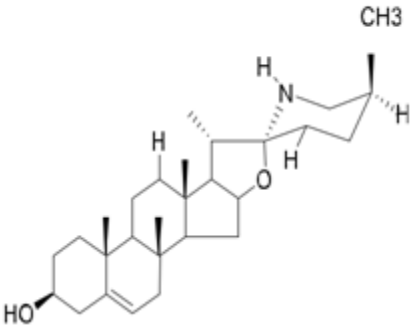
Diosgenin (Suthar *et al.*, 2008)

2.5.1.2 Steroidal alkaloids

Studies show that sixty three steroidal alkaloids are present in the genus *Solanum* of these Solamargine is the major steroidal alkaloid component of the *Solanum* plant found in 18 species (Kaunda & Zhang, 2019). Steroidal alkaloids exhibited various bioactivities which include; antibacterial (Al-Rehaily *et al.*, 2013), antidiabetic, anti-inflammatory, CNS depressants (Jayanthy *et al.*, 2016; (Zhao *et al.*, 2018), spasmolytic, trypanocidal (Kaunda & Zhang, 2019) schistosomicidal (Alsherbiny *et al.*, 2018) among others.



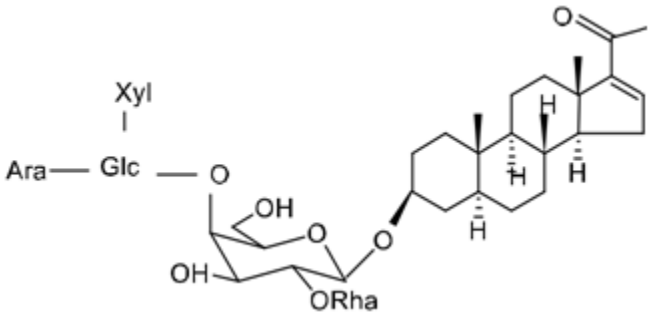
Solamargine (Ding *et al.*, 2013)



Solasodine (Suthar and Mulani 2008)

2.5.1.3 Pregnane Glycosides

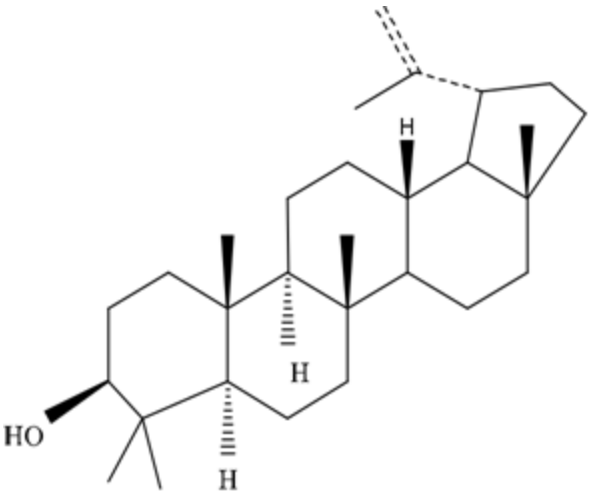
Genus *Solanum* comprise of pregnane glycosides which are biosynthesized in small amounts from steroidal glycosides. Among the compounds isolated *S. nigrum* include; hypoglaucin H, Solanigrosides A and B (Zhou *et al*., 2007). These compounds have demonstrated anticancer properties.

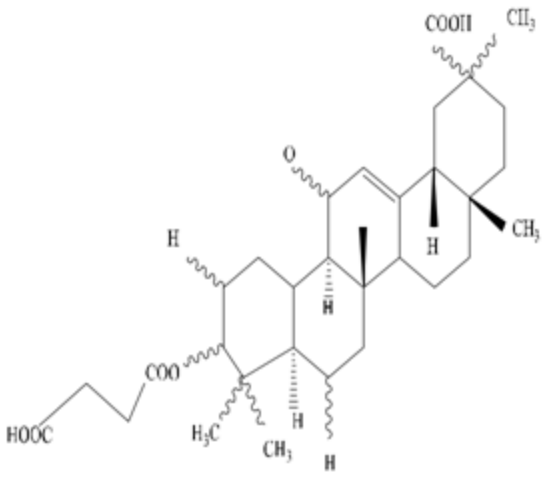


(Solanigroside A (Zhou *et al*., 2007)

2.5.1.4 Triterpenes

Among the triterpenes isolated from the *Solanum* spp., lupeol was the most dominant in several species such as *S. cathayanum*, *S. schimperianum*, (Patel *et al*., 2018) *S. spirale*. The *Solanum* triterpenes have showed to have anticancer properties, for example they showed activity against oral cavity cancer (Keawsa-Ard *et al*., 2012).

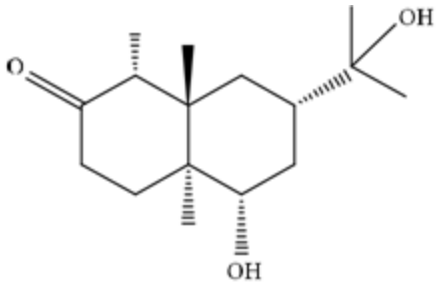


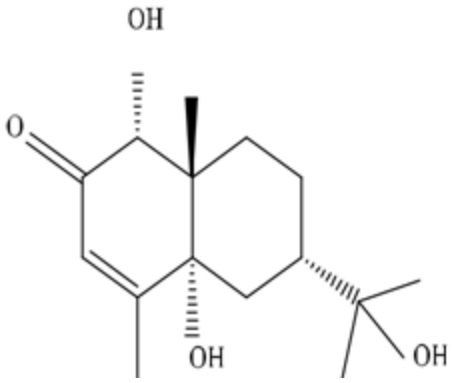


Lupeol (Corrêa *et al*., 2009) Carbenoxolone (Xie *et al*., 2008)

2.5.1.5 Sesquiterpenes

Majority of these compounds were derived from the leaves and fruits of *S.lyratum* (Yao *et al*., 2013) *S.erianthum* (Nie *et al*., 2014) *S.septemlobum* and roots of *S.torvum* (Yuan *et al*., 2016); Kaunda & Zhang, 2019). The bioactivities noted include; anticancer and antifungal activities.

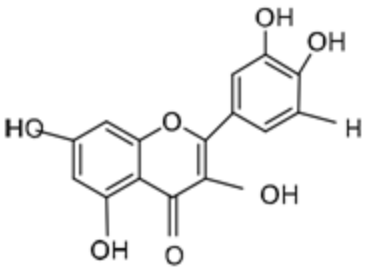




Lyratol G (Nie *et al*., 2014) Solajiangxin A (Yao *et al*., 2013)

2.5.1.6 Flavonoids

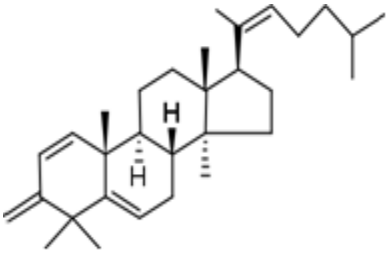
Seventy-two flavonoids that were isolated from the genus showed kaempferol and quercetin as the primary flavonoids (Vanaja *et al.*, 2014) (Examples of glycosylated flavonoids present include; afzelin, came l-liaside C from *S. erianthum, astragalin, kaemferol* (Ramos & de Oliveira, 2017). These flavonoids have displayed various bioactivities which include, anti-cancer, antiviral, hepatoprotective, anti-depressants and cytotoxity effects against breast cancer cell lines (Kaunda & Zhang, 2019).



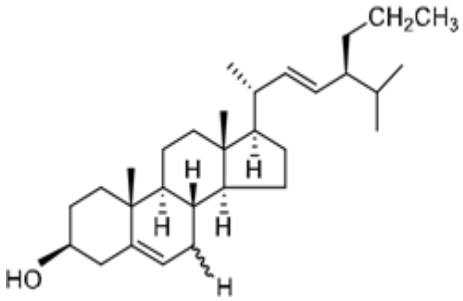
Quercetin (Magar & Sohng, 2020)

2.5.1.7 Sterols

The sterols obtained from the genus *Solanum* include; daucosterol, stigmasterol, clistol G, capesterol, and capsisteroid which have shown antiplasmodial, anticancer and antifungal activities (Petreanu *et al.*, 2016).



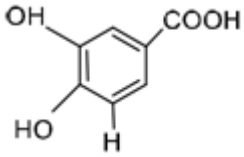
Nigralanostenone (Connolly & Hill, 2010)



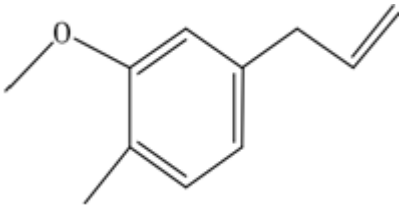
Stigmasterol (Badawy *et al.*, 2013)

2.5.1.8 Phenolic compounds

Phenolic compounds have showed antibacterial, antihypertentive, anticancer and antidiabetic activity (Elizalde-Romero *et al*., 2021).



Protocatechuic acid (Wang *et al*., 2010)



Eugenol (Gong *et al.*, 2016)

2.6 Techniques of measuring obesity

There are different techniques often used to calculate and approximate fat distribution and body composition. These vary from the uncomplicated and useful anthropometric measurements as shown below.

2.6.1 Body Mass Index (BMI)

The body mass index (BMI), which calculates weight in relation to height, is typically used to categorize adult obesity. It is defined as weight in kilogrammes divided by height squared in metres (kg/m2) (Organization, 2015b). Table 2.1 shows the BMI classification, developed based on the European international standards for adults.

Table 1: BMI Classification

Classification	BMI	Remarks
Underweight	<18.5	
Normal range	18.5–24.9	Average
Overweight	Classification	
Pre-obese	25.0–29.9	Increased
Obese I	30.0-34.9	Moderate
Obese II	35.0–39.9	Severe
Obese III	≥40.0	Very severe

2.6.2 Waist – Hip ratio (WHR)

Waist to hip proportion is not only an obesity marker but also a possible indicator for other health conditions (Borel *et al*., 2018). WHR is defined as abdominal obesity above 0.90 for males and 0.85 for females. It has been reported as a better predictor for cardiovascular disease; women and men with WHRs above 0.8 and 1.0 respectively have a greater health risk due to the distribution of fat. WHR = Waist circumference / Hip circumference (Bacopoulou *et al*., 2015).

2.6.3 Air Displacement Plethysmography

This technique has been used for nearly a century in assessing human body composition. However, it had not been developed into a viable system until mid 1990's. In adults and children, air displacement plethysmography has confirmed its success for body-composition measurements. It is non-invasive, quick, comfortable and safe (Roggero *et*

al., 2012). Displacement of Air Plethysmography is an indirect technique that estimates body density by measuring mass and volume in addition to tracking minute changes in fat mass and free fat mass. The ratio of body mass to volume yields an estimate of both fat and fat-free mass (Warolin *et al*., 2012).

2.6.4 Biometric Impendance Analysis (BIA)

Bio-Impedance measures electrical signals as they pass through the water in the body, lean mass and fat. This method is quite simple and measures the entire water in the body, lean mass of individuals and fat mass. Although, it is not dependable compared with the gold standards of submerged weighing, BIA is of value in examining patient's improvement (Faria *et al*., 2014; Khalil *et al*., 2014). Bio-impedance testing involves introducing an alternating electrical current into the body and measuring the body's resistance to the current flow. Lean mass produces less resistance (impedance) than body fat (adipose tissue), which slows the current's flow (de-Mateo-Silleras *et al*., 2019).

2.6.5 Magnetic Resonance Imaging (MRI)

This is an accurate tool for measuring organs, abdominal fat quantity and distribution (Hu *et al*., 2011). It helps researchers to investigate the preventive and therapeutic measures against obesity and its comobidities. Magnetic resonance imaging is the most desired method for assessing body composition since it is non-invasive, safe, provides repeatable, unmatched 3-D visualization and also applicable to all subjects of all ages (McComb *et al*., 2015). However, this method is relatively expensive and requires extensive analysis. The factors that influence MRI intensities include; spin density, tissue-specific, repetition time of the pulse sequence and flip angle (Hu *et al*., 2011). Fat quantification can be done

using MRI imaging techniques. These comprise Dixon techniques and MR spectroscopy. These methods quantify bodily organ fat as well as fat in the skin and abdomen. In addition, it also measures ectopic fat accumulation, muscle fat infiltration and muscle volumes (Mitra *et al.*, 2017).

2.6.6 Hydrodensitometry

Underwater weighing or hydrostatic body composition analysis is also known as hydrodensitometry (Kasper *et al*., 2021). It is a technique for evaluating body mass per unit volume. It has been considered long enough as a gold standard for measuring body composition, however, it is expensive (Kasper *et al*., 2021). By determining your body weight on land and underwater, the hydrostatic approach is used. Test administrators can determine your body density and body fat percentage using the difference between these two readings. Inversely, a comparatively heavy underwater weight suggests a reduced body fat proportion (Collier *et al.*, 2020).

2.7 Microbiome

Microorganism community in the body is known as microbiome, this name was coined by Joshua Lederberg a Laurete who described ecological community, pathogenic microorganism and commensal (Del Frari & Ferreira, 2021). The human body consists of hundreds of trillions (10^{14}) of microbes thus out numbering the eukaryotic cells which are 60 trillion (Okeke *et al*., 2014). These microorganisms are found in the gastro intestinal tract from mouth to rectum. However the largest colonization of microbes is mainly on the colon (10^{11}- 10^{12}) (Scotti *et al*., 2017). Throughout the year's microorganisms have found ways of living in the body symbiotically assisting each other to survive. There are diverse kinds of microbiomes ecosystem these include; skin microbiome, urogenital,

gastrointestinal, oral and each is either composed of viruses, bacteria and archea (Belizário *et al*., 2018). Human microbiome in a given environment has impact on health and disease either through indirect mechanisms or through exposure to pathogenic or beneficial microorganism (Flandroy *et al*., 2018). It is also unique to individuals and rapidly spread into the surrounding environment especially to persons sharing the same space. In addition there are factors that lead to its diversity this include; diet, antibiotic use, geography, psychological and physical health, culture among others (Lax *et al*., 2015; Sharma *et al*., 2019).

2.7.1 Gut Microbiome

The Gut microbiome is classified to almost 1000 different species but to date only about 52 phyla have been identified, of these those that colonize the colon are 10 with only 2 phyla dominating (Shin *et al*., 2015). Diverse microbial composition occurs just before birth and is normally transmitted from mother to child, this is evident through the meconium (Yassour *et al*., 2018). The first 3 to 5 years of early childhood's, microbiota is similar to that of adults. A healthy human gut microbiome is dominated by either Firmicutes (Gram positive bacteria) or Bacteridetes (Gram negative bacteria), Actinobacteria (gram positive), Verrucomicrobia (Gram negative), proteobacteria (Gram negative) and Fusobacteria (Gram negative) (Kachrimanidou & Tsintarakis, 2020a; Doaa *et al*., 2016; Wesonga *et al*., 2016). The colon being the most predominated with the microbes have more than 90% of Bacteriodites such as (*prevotella* and *Bacteroides*) and Firmicutes such as (*Lactobacillus Clostridium, Enterococcus and, Ruminococcus*). The gut microbe's aim in the small intestine is to metabolize dietary fibers that cannot be processed by the available human enzymes (Arora & Sharma, 2011) while in the large

intestines microbes present include; *Lactobacillus*, *Actinobacteria* (*Bifidobacterium* spp) and clostridial clusters IV, XIVa. These microbiotas facilitate the fermentation process of dietary plant polysaccharide (fibers), indigestible oligosaccharides, intestinal mucin and non- digested proteins in order to produce short chain fatty acids such as butyrate propionate and acetate (Flint *et al*., 2012). Additionally, the gut microbiota plays a role in the digestion of food as well as in the prevention of pathogen invasion by promoting colonization resistance, activating adaptive immunity, producing vitamin K and B, preserving intestinal epithelial integrity and homeostasis, and improving the function and motility of the gastrointestinal tract (GIT) (Zhang *et al*., 2015). Bacteriodites break down non digestibles carbohydrates and glycans while Firmicutes especially of the genus *Clostridium* help in fermenting aminoacids and degrading polysaccharides (Flint *et al*., 2015).

2.7.2 Oral Microbiome

One of the body's dynamic ecosystems is the oral cavity, previous studies indicate that it comprises of 50 -100 billion bacteria and almost 700 bacterial species have been identified through 16S RNA (Robinson *et al*., 2010; Xu *et al.*, 2015). The oral microbiota of upto 80% comprise of 200 species of Proteobacteria, and Firmicutes along with Bacteroidetes, Actinobacteria and Fusobacteria totaling to 95 % of all the mouth microbiota (Chen *et al.*, 2010; Xu *et al*., 2015). Further diversity of microbiome is varied in the different parts of the mouth which include the gingiva, tooth surface, soft and hard palate and on the tongue (Xu *et al*., 2015). Studies indicate that an overgrowth of specific microbiota is associated with systemic disease (Chen & Jiang, 2014) for example dental carries is associated with saccharolytic (sugar metabolizing bacteria) such as

Lactobaccillus and *Streptococcus* while proteolytic bacteria (protein metabolizing) such as *Pophyromonas* and *Prevotella* is associated with halitosis and periodontitis (Takahashi, 2015). Other factors that lead to increase in certain oral bacteria include; smoking (Morris *et al*., 2013), cancer (Atanasova & Yilmaz, 2014) and artherosclerosis among others and hence leads to Pophyromonas gingivalis (Al Khodor *et al*., 2017a).

Disease occurrence is mainly as a result of microbial dysbiosis. These explains why individuals are likely to incur more severe forms of illness than others. Dysbiosis is the change in structure and composition of human microbiota at a given site. Recent research have revealed that the microbiome, despite not having a cause and effect relationship with microbial stability and alignment, it is a major contributor to many diseases, particularly non-communicable diseases like diabetes (Dunne *et al*., 2014), obesity (Bradlow, 2014), cardiovascular diseases (Dinakaran *et al*., 2014), cancer (Rogers *et al*., 2014), asthma, inflammatory bowel disease among others (Major & Spiller, 2014). Studies also show that metabolites from the microbiota cause chronic kidney disease, resulting in the diversity and richness of the microbiota.

2.8 Measurements used in Microbiome analysis

2.8.1 Alpha diversity

Alpha diversity is a measure of the ecological community's evenness (the distribution of abundances among the groups) and richness (the number of taxonomic categories), or both. Alpha diversity is also defined as the variability of species within a habitat or intra community diversity, for example, a community with an even number of members from different species will have a higher evenness index, whereas a community with fewer species overall will have a lower evenness index (Willis, 2019). There are many factors

that affect the alpha diversity of a community, these include; change in diet, age, temperature, time, biogeochemical conditions, pathogen infection, anthropometric factors among others (Willis *et al*., 2017). The first approach in assessing the differences in microorganisms between environments was done through amplicon sequencing analysis. Alpha diversity occurs when you compare environments to identify differences in microbial taxa. The overall number of species present in the community is known as richness index (Thukral, 2017).

In microbial studies one is interested in various aspect of diversity which include; relative abundance, species evenness and species richness. Relative abundance describes the commonness or rareness a specie is relative to another while specie evenness describes the equality of abundant species in an environment. Specie richness describes how many unique species are present in an environment. In this study, the gut microbiota is typically characterized by the dominance of the phylum campylobacter. This makes species evenness an important tool to look at when considering alpha diversity. There are different types of diversity indices; these include information statistics and dominance indices. Information statistics assumes that the species are detected and randomly sampled, it combines both evenness and richness in a single measure. This measure could be a bit problematic since it makes it difficult to determine the source of differences either in richness or diversity. An example of information statistics includes Shannon diversity. Dominance indices are weighted according to abundance for reducing the effect of rare species with few representatives on the diversity score.

2.8.2 Diversity Indices

There are different indices used in diversity analysis which include; Shannon- Weaver and Simpson diversity. These diversity indices give more inference about the composition of the community rather than the richness or evenness of the species (Kim *et al*., 2017). The Shannon weaver index gauges the average level of uncertainty in classifying a randomly selected species. The value increases as the distribution of individuals among the species become even and as the number of species increase. On the other hand, Simpson index indicates dominance in species and reflects the probability of two individuals which belong to the same species being randomly chosen *(*Morris *et al*., 2014). Shannon index increases from 0-1 and is inversely proportional to the diversity, however, Simpson index ranges from 0-1 but the higher the value the greater the diversity (Hossain *et al*., 2017; Lemos *et al*., 2011).

2.8.3 Beta Diversity

The measurement of specie composition variation between samples is called beta diversity (Han *et al*., 2018). It is a simple ratio between gamma and alpha diversities. There are a lot of dissimilarities or ecological distances for measuring the closeness of two microbial compositions (Faust *et al*., 2012). Comparing the variations in therapy between samples is one of the objectives of this research. It is a common method for comparing communities and is also used to compute the distance between all samples (Hugerth & Andersson, 2017). The true measure of distance is called metric and for it to be a true metric it has to fulfill three requirements which include; symmetry, should satisfy the triangular inequality condition and the distance should be the same independent of the directionality measure. For example; in microbial communities

dissimilarities between community A and B plus dissimilarities between community A and C is not necessarily greater than between communities B and C (Greenacre & Primicerio, 2013). Two common dissimilarity measures used for microbial study include Bray Curtis and Unifrac (Bray & Curtis, 1957; Lozupone *et al*., 2012). These measures do not require equal variances thus their transformation to proportions is considered more suitable. In this study Bray Curtis was used for beta diversity and Simpson measure.

CHAPTER THREE

ANTI- OBESITY EFFECTS OF DICHLOROMETHANE - METHANOL LEAF EXTRACT of *Solanum nigrum* IN HIGH –FAT FED RATS

Abstract

Solanum nigrum (Subsp *villosum)* biosynthesizes various phytochemical compounds which are known to have various pharmacological activities that treat diabetes type 2, cardiovascular diseases among other metabolic diseases.

Materials and Methods

To assess the anti-obesity effects of *Solanum nigrum* (Subsp *villosum)* on high-fat-fed diet rats, Sprague Dawley male rats (n=35) of weights 160-180g were assigned randomly into seven groups comprising n=5 rats each. Each group was fed for 11 weeks as follows: normal control (normal chow rat feed); high-fat diet control (HFD); HFD and standard drug (Orlistat 30mg/kg bw); HFD and methanolic extract 150mg/kgbw; HFD and methanolic extract 300mg/kgbw; HFD and dichloromethane extract 150mg/kgbw; HFD and dichloromethane extract 300mg/kgbw. Body mass index and food intake were monitored per week and an oral glucose tolerance test was measured in weeks 5 and 10. On the final day of the laboratory work, adipose tissue, liver weights, lipid profiles, liver function tests, and a phytochemical analysis of *Solanum nigrum* (Subsp *villosum)* were performed.

Results

There was a significant increase in body mass index (BMI) of high-fat diet control group compared to rats administered with leaf extracts of *Solanum nigrum* (Subsp *villosum)* which showed a reduction in BMI. Both low dose of dichloromethane (150mg/kgbw) and

high dose of methanol extracts (300mg/kgbw) showed a better reduction in BMI than the other methanolic and dichloromethane treatment groups. A significant decrease ($p < 0.05$) on cholesterol, low-density lipoprotein-cholesterol and triglycerides was observed among the rats administered with *Solanum nigrum* (Subsp *villosum)* extracts compared to those of HFD control. Moreover, the HFD negative control group significantly increased adipose tissue and liver weights compared to the normal control, positive control and DCM- methanol treatments groups ($p<0.05$). On the other hand, *Solanum nigrum* (Subsp *villosum)* also normalized the hepatic enzymes and decreased glycemic levels of DCM-methanolic treatment groups. However, food intake among the groups showed no significant difference ($p>0.05$). Qualitative analysis of *Solanum nigrum* (Subsp *villosum)* leave extracts indicated the presence of flavonoids, alkaloids, saponins, steroids and phenols bioactive compounds which are associated with anti-obesity.

Conclusion: These results validate the use of *Solanum nigrum* (Subsp *villosum)* in the controlling of obesity.

3.1 Introduction

Obesity is the phenotypic appearance of abnormal fat buildup in the adipose tissue that alters the health of an individual hence causing an increase in morbidity and mortality (Zhou *et al*., 2021). It is linked with various contra indications such as cardiovascular diseases, diabetes, chronic morbidities such as stroke, sleep apnea, osteoarthritis, hypertension among others (De Lorenzo *et al*., 2019). The adipose tissue phenotype in obesity is regulated by genes, diet, or both. Obese persons are either hyperplasia (many small adipocytes) or hypertrophy (Few large adipocyte) and both lead to adipose tissue expansion (Vishvanath & Gupta, 2019). Previous research studies have indicated that hypertrophy correlates with diet while hyperplasia is mainly genetics.

In hypertrophy the adipocytes outgrow in size, reaching non- physiological limits thus are incapable of functioning as energy storing organ, develops apoptosis and changes their endocrine function by becoming resistant to insulin (Rescan *et al*., 2017). Resistance to insulin is as a result of ectopic translocation of free fatty acids and adipose tissue inflammation. The excess fat shifts to the non-adipose tissues where they employ toxicity leading to dysfunctioning organs. These organs encompass liver, β cells of the pancreas, skeletal muscles and heart. Lipid toxicity is caused by fat oxidation impairment in tissues where free fatty acids enter skeletal muscles increasing triglyceride content and reducing glucose uptake. Since it's entirely dependent on insulin, it requires insulin receptors a phosphorylative cascade and Glut- 4 transporters (Ahmad *et al*., 2021). Glucose is taken up by liver, where it is stored in form of glycogen and TG, it is then catabolized to free fatty acid thus releasing glucose when required (Malone & Hansen, 2019). Increase in TG levels leads to slight increment in TC and a decrease in HDL-c. Furthermore, hepatic

lipase metabolizes the low-density lipoprotein cholesterol (LDL-c) abundant in TG converting it to small LDL-c hence promoting dyslipidemia (Toth, 2016).

Recent global approach to obesity are focusing on modifying lifestyle and diet of individuals by restricting calorie intake, reducing physical inactivity and increasing the consumption of natural products that have been proven to have compounds, that have health benefits and can prevent disease. Multiple combination of compounds has synergistic effects that would increase their bioavailability and actions on various molecular targets hence offering benefit over standard drugs (Mohamed *et al*., 2014b). These compounds are mediated by regulation of various pathways that include; decreased lipogenesis, lipid absorption, differentiation and proliferation of pre-adipocytes, and consumption and increase in expenditure of energy (Ahmad *et al*., 2021).

In this chapter anti-obesity effects of DCM- methanolic extracts of *Solanum nigrum* (Subsp *villosum)* was determined in high fat fed diet rats.

3.2 Methodology

3.2.1 Collection and Preparation of Plant Materials

Fresh leaves that were sixty days old were collected from limuru subcounty (-1.115365, 36.659386) Kiambu County. The leaves were carried to the University of Nairobi's biological science department herbarium in plastic bags for plant identification and validation by a recognized taxonomist, and the voucher specimen was deposited there as well (Voucher specimen number (KWNUON2019/001). The leaf samples were then spread to dry under a shade and later grounded into powder using an electric mill. The resulting 2.5 kg of powdered material were then stored at room temperature away from direct sunlight in dry plastic containers ready for extraction.

3.2.2 Extraction of Phytochemicals

Extraction was done in Chemistry Department Laboratories at the University of Nairobi using the protocol by (Shalini & Sampathkumar, 2012). The ground plant material was first immersed in 100 percent hexane for 24 hours, then in 100 percent dichloromethane (DCM) for 24 hours, filtered, and immersed again for another 24 hours, and finally in 100 percent methanol at room temperature for 24 hours, followed by another 24 hours of immersion, respectively.

Hexane, dichloromethane and methanol solvents filtrates were filtered using whatman number 1 filter paper and later concentration done by rotary evaporation at 68^{o}C, 39^{o}C, 64^{o}C respectively according to their boiling points. In preparation for the bioassay, the concentrate was weighed and stored at 4^{o}C.

3.3 Experimental Design

3.3.1 Laboratory Animals

A total of 35 male Sprague dawley rats weighing 160 - 180g were purchased from vet laboratories Kabete and transported to the University of Nairobi Biochemistry department animal house. The rats were kept for one week in standard cages at normal laboratory conditions (25± 2ºC, 12 h light and 12h dark cycle) before the start of the experiment in order to acclimatize. The rats were given the standard pellet and supplied with water ad libitum. Guidelines by institutional review committee (IRC) of Institute of primate research on animal models were followed ISERC/06/19.

3.3.2 Induction of Obesity

To induce obesity, rats in group II-VII were fed on high-fat diet every day for eleven weeks. The high fat diet (HFD) was prepared by addition of 30 g of fat (Seagull* frying fat, Kapa oil refineries, Kenya) to 100 g of rat chow pellets (Fat: 10%, Protein: 20% and Carbohydrate: 70%) (Unga Feeds Limited, Nairobi). To improve the palatability of HFD 0.8% of monosodium glutamate (MSG) (Oshwal Flavours Limited, Nairobi, Kenya) was added. This was done by heating the mixture for 20 minutes with constant mixing to absorb the fat into the feed (Mutiso *et al*., 2014).

3.3.3 Biological Assay

The male Sprague dawley rats were randomized into groups of five rats each as follows: Group I (normal control), Group II (negative control) was induced obesity with (HF-Research diets) and was given oral administration of water (0.1 ml/rat). Group III (positive control) was induced obesity (HF research diet) and received standard drug

orlistat at a dose of 30mg/kgbw, Groups IV to VII (experimental groups) were induced obesity (HF- research diet) and received DCM dose extracts of *S. nigrum* (Subsp *villosum*) and Methanol dose extracts of *S. nigrum* at 150 mg/kgbw and 300mg/kgbw respectively. To determine the dose extract, a pilot investigation was previously carried out.

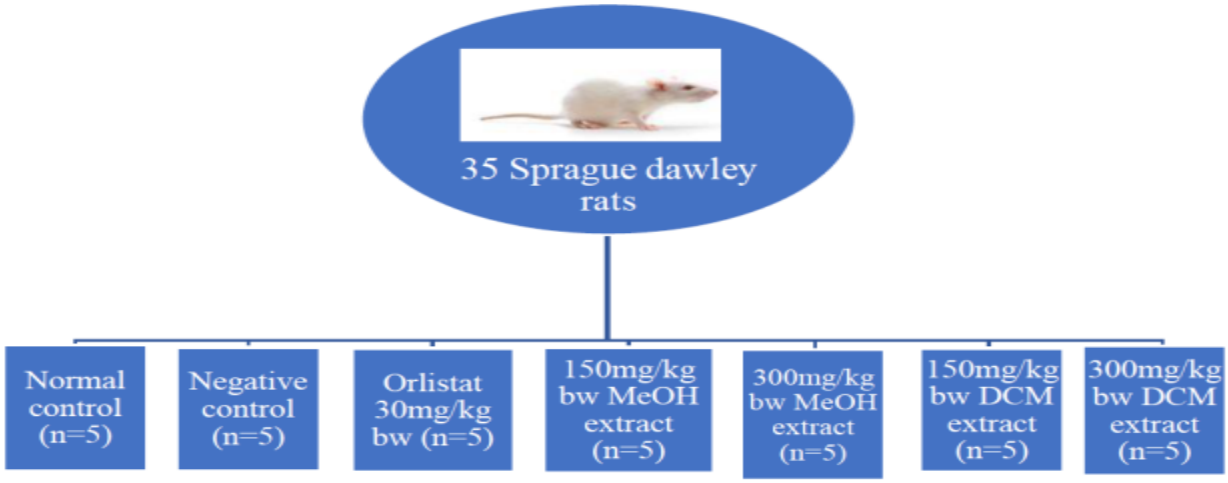


Figure 2: Experimental groups

3.3.4 Body Mass Index Determination

To measure the body mass index of rats. The rat lengths (naso-anal length) and weights were examined every week for 11 weeks using a ruler and an electronic balance respectively. To verify the rats body mass index; Lee index was calculated, as follows:

$$\text{Lee index BMI} = \left(\sqrt[3]{Body\ weight} \Big/ Nasal - anal\ length\ (cm) \right) \times 1000$$

Rats with lee index ≥ 310 mass index were considered obese (Lee, 1929).

3.3.5 Food Consumption Determination

Food consumption was measured daily during the experimental period. The animals were given 150g of rat chow per cage in the morning and the food remaining weighed after 24 hours before the administration of the extract (Arika *et al*., 2019b).

3.3.6 Oral Glucose Tolerance Test

Oral glucose tolerance test was determined on week five and week ten of the experimental period using a glucometer (onco-plus). The animals were fasted overnight (12h) prior to the test and blood samples obtained by snipping the lateral tail vein of the rat (Chege *et al*., 2019). To minimize pain Lidocaine was applied on the tail prior to the procedure (Lee & Goosens, 2015). The blood sugar was assessed using a glucometer at baseline and immediately a loading dose of glucose (2g/kgbw) was administered to each rat by oral gavage. The blood glucose samples were then collected at 30, 60, 90 and 120 minutes after oral administration of the loading dose of glucose. Areas under the curve (AUCs) were then calculated (Chege *et al*., 2019).

3.3.7 Serum Sample Preparation

The rats were put to death using carbon dioxide on the 77th day to ease their suffering during sacrifice. The animals were then dissected by removing the fur in order to draw blood from the cardiac puncture. The Blood samples (5mls) were then transferred into plain micro vacutainer tubes and centrifuged at 2500 rpm for 15 minutes for serum collection. The serum collected was used for biochemical analysis.

3.3.8 Biochemical Assays

Biochemical parameters including low density lipoprotein (LDL-c), triglycerides, high density lipoprotein (HDL-c), total cholesterol, Alanine amino transferase (ALT), Gamma glutamyl transferase (GGT), Alkaline phosphatase (ALP) and were determined using automated biochemical analyser (Olympus 640) according to the standard operating procedures (Sops) written and maintained in the department of laboratory medicine, Kenyatta National Hospital (Arika *et al*., 2019a). Values of Alanine amino transferase above 55 IU/L, alkaline phosphatase above 260 IU/L and Values of Gamma-glutamyl transferase above 40 IU/L were considered abnormal.

3.3.9 Liver and Adipose Tissue Excision

After euthanization the liver and the adipose tissue were excised and weighed using a weighing scale of (WANT Balance Instrument Co. Ltd, China).

3.3.10 Qualitative Phytochemical Screening

A qualitative phytochemical screening was performed on the crude extract to determine whether or not bioactive chemicals were present. The secondary metabolites included flavonoids, alkaloids, steroids, saponins, diterpenes, tannins, phenolics, terpenoids and anthroquinones.

3.3.10.1 Terpenoids (Salkolski Test)

A gram of each extract was mixed with 2ml of chloroform and 1ml petroleum ether /1ml of ethyl acetate. Three milliliters (3ml) of concentrated sulphuric acid was added alongside to form a layer. Reddish brown coloration indicated presence of terpernoids (Zunjar, 2017).

3.3.10.2 Saponins (Froth test)

A test tube containing one gram of extract was filled drop by drop with two milliliters of distilled water and two milliliters of sodium bicarbonate solution. The mixture was agitated, and 15 minutes of foaming revealed the presence of saponins (Akinpelu *et al*., 2014).

3.3.10.3 Anthroquinones

For a few minutes, one gram of the extract was boiled in 10% HCL in a water bath. It was then filtered, chilled, and the filtrate heated following dropwise additions of 1 ml of 10% ammonia and 1 ml of chloroform. The presence of anthraquiones was indicated by the rose pink coloration (Tiwari *et al*., 2016).

3.3.10.4 Flavonoids (Sodium Hydroxide Test)

Two milliliters of diluted sodium hydroxide were added to one gram of extract. Golden-yellow precipitate showed flavonoids were present (Tongco *et al.*, 2014)**.**

3.3.10.5 Steroids

A layer was created by dissolving one gram of leaf extract in two milliliters of chloroform and then adding three milliliters of concentrated sulfuric acid to the test tube's sidewalls. Reddish brown color meant presence of steroids (Dhawan & Gupta, 2017).

3.3.10.6 Phenols

One milliliter of ferric chloride solution was mixed with 1 gram of each extract. The presence of phenols was identified by a blue to green color formation (Jaradat *et al.*, 2015).

3.3.10.7 Tanninns

Two drops of 5% iron chloride and one milliliter of distilled water were added to one gram of extract. Tannin was indicated by the presence of blue-black pigment (Mailoa *et al*., 2014).

3.3.10.8 Diterpenes

A gram of extract was dissolved in water and later three drops of copper sulphate solution added. Presence of diterpenes was determined after the color changed from blue to emerald green (Osama & Awdelkarim, 2015).

3.3.10.9 Data and Statistical Analysis

Experimental data on organ weight, body mass index, glucose levels, food consumption and biochemical analysis was tabulated/organized on Microsoft Excel spread sheets for statistical analysis. One-way analysis of variance (ANOVA) and a post hoc Tukey test was used to determine whether there are significant differences between the treatment groups values of $p \leq 0.05$ were considered significant.

3.4 Results

3.4.1 Body Mass Index

The methanolic leaf extracts of *Solanum nigrum* (Subsp *villosum)* caused significant decrease in the body mass index (BMI) of high fat fed rats (HFD) (Table 2). The rats treated with methanol extracts dose of 150mg/kg/bw had a non-significant decrease in BMI compared to the normal control (placebo) at week 3-9 (302.30 – 303.54) ($p>0.05$) but showed a significant decrease at week 11 (301.50) ($p<0.05$). However, the rats administered with methanol extracts dose of 300mg/kgbw had a significant decrease in body mass index with the normal control (placebo) at week 5-11 (298.70 - 295.16) ($p<0.05$). The body mass index of rats administered with extracts at a dose of 150mg/kgbw and 300mg/kgbw had a similar trend with that of rats treated with orlistat (positive control). Moreover, BMI for the rats treated with methanol extracts dose of 150mg/kg/bw and 300mg/kgbw were nonsignificantly different with the rats treated with orlistat (positive control) at week 5-11(301.33 – 298.20) ($p>0.05$). The rats treated with methanol extract dose of 150mg/kgbw and 300mg/kgbw had a significant decrease in BMI compared to the rats on high fat diet (negative control) at week 5-11(308.57 – 313.08) ($p<0.05$).

The dichloromethane (DCM) *Solanum nigrum* (Subsp *villosum)* leaf extracts also caused significant changes in the BMI of high fat fed rats. The BMI of dichloromethane leaf extracts dose of 150mg/kgbw showed a significant decrease in BMI compared to the normal control (placebo) at week 5-11 (301.87- 297.84) ($p>0.05$). However, the BMI of rats treated with DCM extracts dose of 300mg/kgbw showed a significant decrease compared to normal control (placebo) at week 5-11 (305.37-298.85) ($p<0.05$). The BMI of rats administered DCM extracts dose of 150mg/kgbw showed a similar trend with that

of rats administered orlistat drug (positive control) and were nonsignificantly different at week 3, 5, 9 and 11($p>0.05$). However, there was a significant decrease in BMI of rats administered 150mg/kgbw compared to the high fat fed rats (negative control) at week 5-11 (308.57-313.08) ($p<0.05$). The body mass index of rats administered DCM extract dose of 300mg/kgbw showed an nonsignificant increase in BMI compared to the normal control at week 3-7 (300.37-304.25) ($p>0.05$), but showed a significant decrease at week 9-11 (304.59 -304.68) ($p<0.05$). The body mass index of rats treated with DCM extract dose of 300mg/kgbw showed a significant increase in BMI compared to the positive control at week 3-9 (301.28- 300.03) ($p<0.05$). The body mass index of rats administered with DCM extracts dose of 300mg/kgbw showed a significant decrease in BMI when compared to the high fat fed rats (negative control) at week 5-11(308.57-313.08) ($p<0.05$).

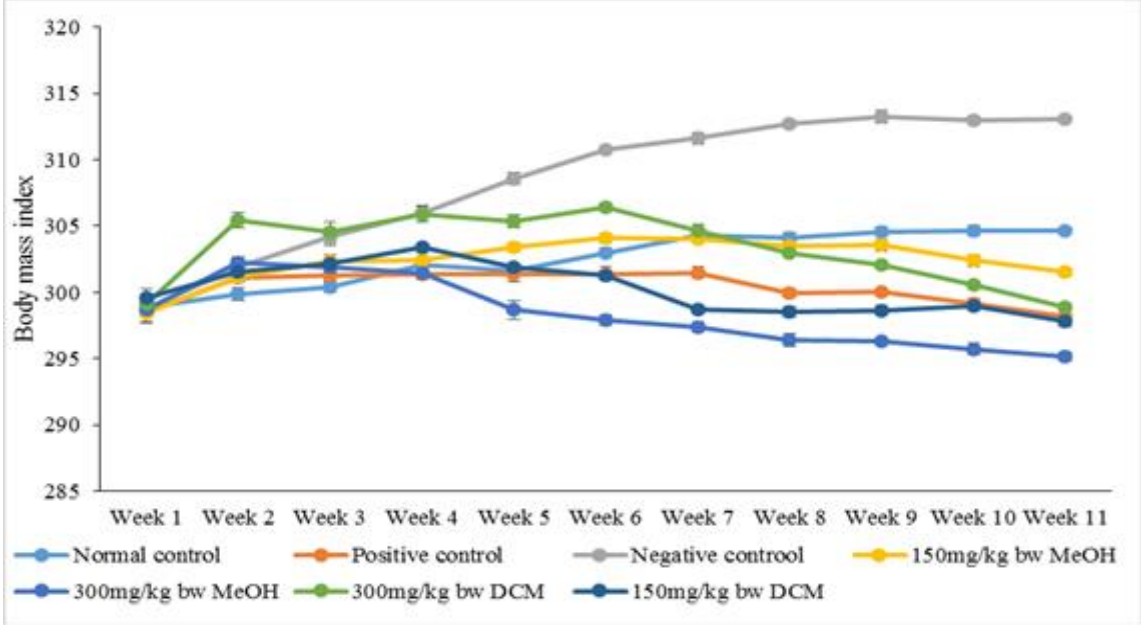


Figure 3: The group administered with dose extract of 300mg/kgbw. MeoH reduced the BMI better than the other experimental groups compared to the negative control

Table 2: Effects of Methanol and Dichloromethane leaf extracts of Solanum nigrum (Subsp villosum) on body mass index on high fat fed rats

Treatment	Week 1	Week 3	Week 5	Week 7	Week 9	Week 11
Normal control	298.89 ±0.52^{a}	300.37±0.38^{c}	301.67±0.40^{c}	304.25±0.43^{b}	304.59±0.34^{b}	304.68 ±0.19^{b}
Positive control (orlistat 30mg/kgbw)	298.54±0.80^{a}	301.28±0.42^{c}	301.33±0.57^{c}	301.49±0.42^{c}	300.03±0.26^{d}	298.20±0.74^{d}
Negative control	298.81±0.60^{a}	304.18±0.61^{ab}	308.57±0.42^{a}	311.62±0.43^{a}	313.24±0.46^{a}	313.08±0.17^{a}
150mg/kgbw. MeoH	298.41±0.71^{a}	302.30±0.52^{abc}	303.41±0.32^{bc}	304.01±0.20^{b}	303.54±0.46^{bc}	301.5±0.26^{c}
300mg/kgbw. MeoH	298.71±0.59^{a}	301.92±0.56^{bc}	298.70±0.71^{d}	297.36±0.42^{d}	296.33±0.20^{e}	295.16±0.31^{e}
300mg/kgbw. DCM	299.14±0.57^{a}	304.52±0.79^{a}	305.37±0.47^{b}	304.68±0.47^{b}	302.04±0.23^{c}	298.85±0.20^{d}
150mg/kgbw. DCM	299.63±0.59^{a}	302.18±0.3^{abc}	301.87±0.22^{c}	298.74±0.14^{d}	298.66±0.34^{d}	297.84±0.43^{d}

Values expressed as Mean ± SEM for five animals per group. Statistical comparison was made within a column and values with the same superscript letter are not significantly different by one-way ANOVA followed by Tukey's post hoc test($p>0.05$).

3.4.2 Oral glucose tolerance test

The methanolic and DCM leaf extracts of *Solanum nigrum* (Subsp *villosum)* showed effects on glucose levels on high fat fed rats. The oral glucose test at week 5 showed no significance changes at 0 mins among the groups ($p>0.05$; Table 3a; Fig 4a) but showed significance difference at 120mins ($p<0.05$; Table 3a; Fig 4a). However, at week 5 the rats did not indicate glucose intolerance. Table 3b shows glucose tolerance test at week 10, the results show that there was a significant change in glucose levels as shown on Table 3b. The glucose level of rats on high fat diet (negative control) were significantly higher than all the treatment groups, normal control and rats treated with orlistat (Positive control) ($p<0.05$; Table 3b; Fig 4b). The results also showed a significance decrease in glucose levels on normal control and rats treated with orlistat (positive control) compared to the negative control ($p<0.05$; Table 3b; Fig 4b). Moreover, at min 30 there was a significant change between the normal control and the rats administered 300mg/kgbw.M, 150mg/kg. D and 300mg/kg. D ($p<0.05$; Table 3b; Fig 4b). There was also a significant decrease in glucose levels of rats given methanolic extracts dose of 150mg/kgbw when compared to the rats on high fat diet (negative control). At min 60 there was a significant decrease on glucose levels between rats administered with methanolic extracts dose of 300mg/kgbw compared to negative control ($p<0.05$; Table 3b; Fig 4b) and a non-significant change on the other groups ($p>0.05$; Table 3b; Fig 4b). However, at min 90 and 120 the rats on high fat diet (negative control) had a significant increase in glucose levels compared to the normal control, positive control and rats administered with methanolic and DCM extracts ($p<0.05$; Table 3b; Fig 4b).

Table 3a: Glucose tolerance test (Week 5)

Treatment	0 min	30	60	90	120
Normal control	3.98±0.48^{a}	6.66±0.56^{b}	5.38±0.35^{b}	4.76±0.25^{a}	4.42±0.19^{b}
Positive control	3.74±0.32^{a}	8.6±0.43^{a}	7.76±0.61^{a}	5.7±0.45^{a}	4.76±0.17^{ab}
Negative control	4.28±0.33^{a}	7.96±0.5^{ab}	7.52 ±0.14^{a}	6.04±0.36^{a}	5.38±0.32^{ab}
150mg/kgbw.MeoH	4.6±0.49^{a}	7.16±0.23^{ab}	7.44±0.16^{a}	6.16±0.44^{a}	5.74±0.35^{a}
300mg/kgbw.MeoH	4.88±0.24^{a}	6.68±0.30^{b}	6.88±0.35^{ab}	5.34±0.27^{a}	5.76±0.22^{a}
300mg/kgbw.DCM	4.5±0.44^{a}	6.78±0.13^{b}	6.52±0.24^{ab}	5.34±0.27^{a}	4.94±0.16^{ab}
150mg/kgbw.DCM	5.5±0.31^{a}	6.9±0.30^{ab}	6.92±0.77^{ab}	6.05±0.27^{a}	5.95±0.35^{a}

Glucose values expressed as Mean ± SEM for five animals per group at minute 0, 30, 60, 90 and 120 on week 5 of the different experimental groups. Bars with a similar superscript letter are not significantly different by oneway ANOVA and Tukey's post hoc test ($P \geq 0.05$)

Table 3b: Glucose tolerance test week 10

Treatment	0 min	30	60	90	120
Normal control	4.12 ± 0.22^{b}	5.66 ± 0.13^{c}	6.14 ± 0.19^{ab}	5.72 ± 0.40^{b}	5.62 ± 0.43^{b}
positive Control	4.16 ± 0.08^{b}	6.46 ± 0.17^{bc}	6.84 ± 0.40^{ab}	5.78 ± 0.63^{b}	5.42 ± 0.58^{b}
Negative control	5.1 ± 0.28^{a}	8.32 ± 0.20^{a}	7.8 ± 0.60^{a}	7.82 ± 0.44^{a}	7.26 ± 0.27^{a}
150mg/kgbw.MeoH	4.54 ± 0.27^{ab}	6.9 ± 0.18^{bc}	6.58 ± 0.35^{ab}	6.12 ± 0.33^{ab}	5.78 ± 0.31^{b}
300mg/kgbw.MeoH	4.7 ± 0.03^{ab}	7.34 ± 0.34^{ab}	5.74 ± 0.15^{b}	5.56 ± 0.21^{b}	5.30 ± 0.10^{b}
300mg/kgbw.DCM	4.74 ± 0.10^{ab}	7.24 ± 0.48^{ab}	6.2 ± 0.39^{ab}	6.0 ± 0.22^{b}	5.42 ± 0.13^{b}
150mg/kgbw.DCM	4.64 ± 0.18^{ab}	7.08 ± 0.35^{ab}	6.18 ± 0.53^{ab}	5.54 ± 0.26^{b}	5.52 ± 0.17^{b}

Glucose values expressed as Mean ± SEM for five animals per group at minute 0, 30, 60, 90 and 120 on week 10 of the different experimental groups. Bars with a similar superscript letter are not significantly different by one -way ANOVA and Tukey's post hoc test ($P \geq 0.05$)

Week 5 OGTT Measurements

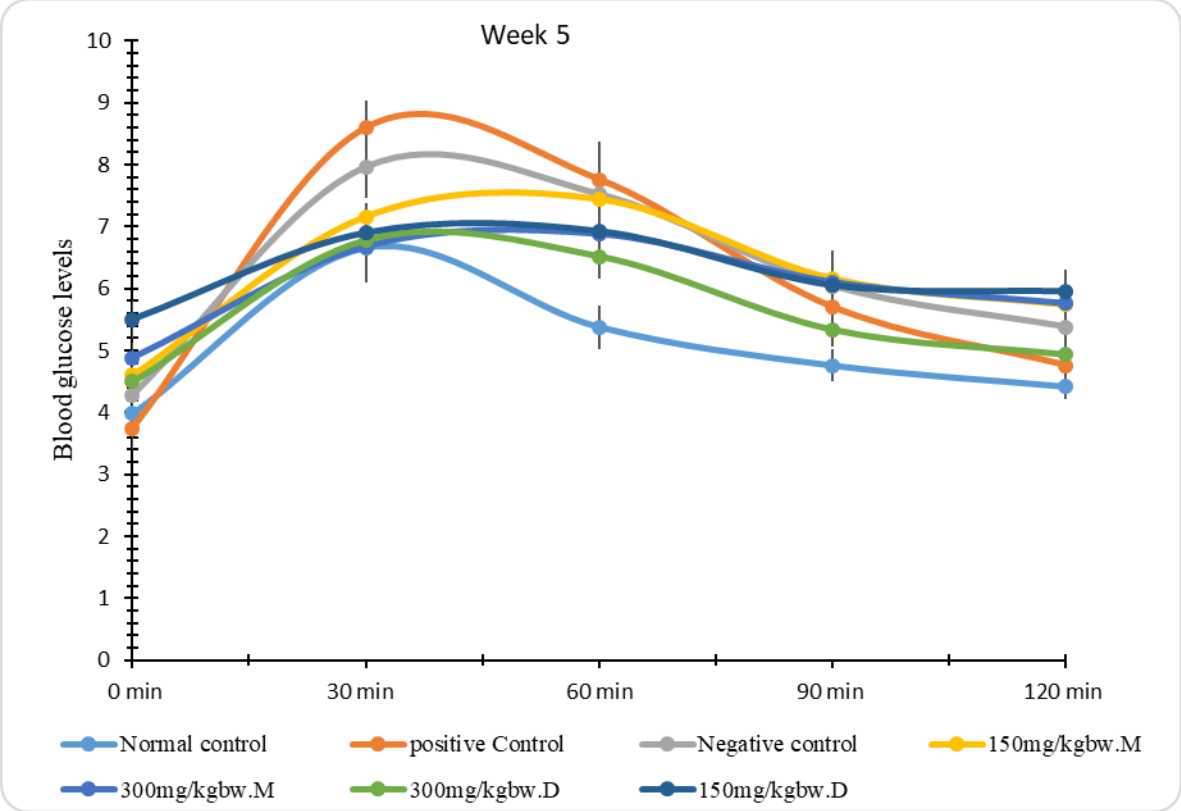


Figure 4a: Glucose levels at week 5

Week 10 OGTT Measurements

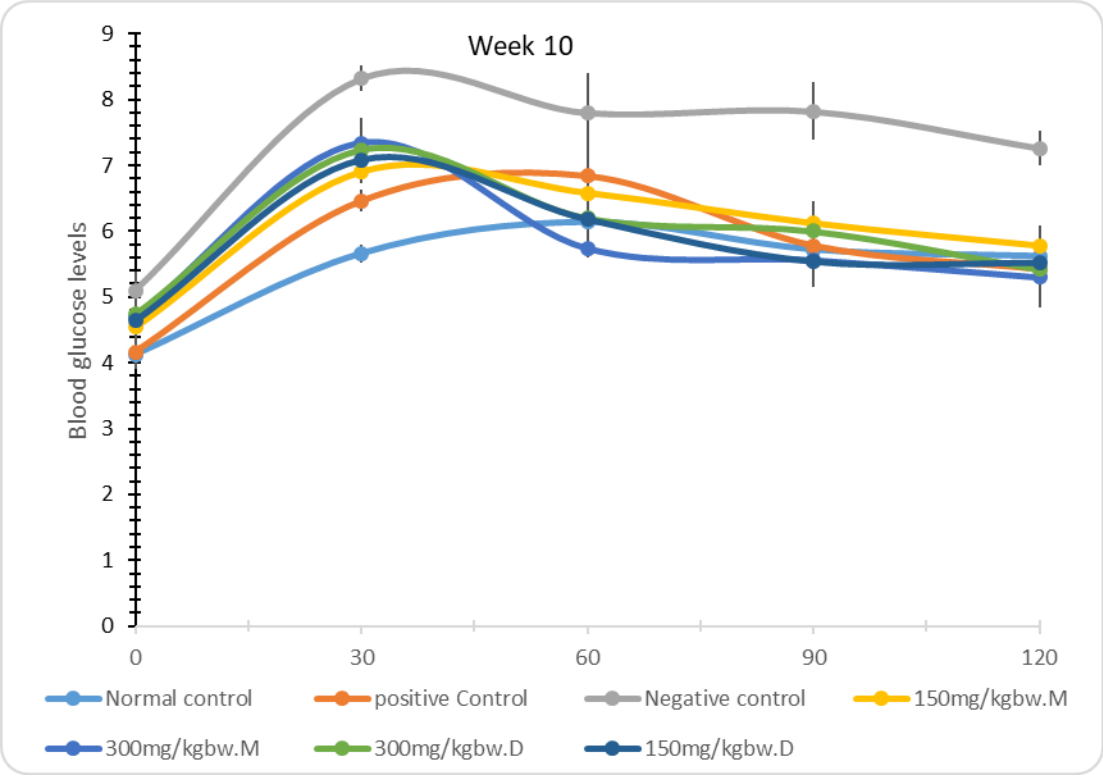


Figure 4b: Glucose levels at week 10

3.4.3 Lipid profiles

Table 4 shows the effects of methanolic and dichloromethane leaf extracts of *Solanum nigrum (*Subsp *villosum)* on total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL-c) and low-density lipoprotein (LDL-c) on high fat fed rats. The rats treated with the methanolic extract dose of 150mg/kg/bw exhibihited a non-significant decrease in TC, HDL-c and LDL-c compared to normal control but showed a non-significant increase in TG compared to normal control ($p>0.05$; Table 4). However, rats administered methanolic extract dose of 150mg/kgbw and 300mg/kgbw exhibited a significant decrease in HDL-c compared to the rats treated with orlistat (positive control) ($p<0.05$; Table 4) and had a non- significant difference in TC, TG and LDL-c compared to the rats treated with orlistat (positive control) ($p>0.05$; Table 4). The rats administered methanolic extracts dose of 150mg/kgbw and 300mg/kgbw showed a non-significant decrease in TC, TG and LDL-c compared to high fat fed rats (negative control) ($p>0.05$; Table 4).

Rats treated with dichloromethane *Solanum nigrum* (Subsp *villosum)* extracts at a dose of 150mg/kgbw and 300mg/kgbw showed a non-significant decrease in TC and a non-significant increase in TG, HDL-c and LDL-c compared to the normal control ($p>0.05$; Table 4). Furthermore, there was a non-significant difference in TC, TG, HDL-c and LDL-c in rats treated with DCM extracts dose of 150mg/kg/bw and 300mg/kgbw compared to rats treated with orlistat (positive control) ($p>0.05$; Table 4). The rats treated with extracts dose of 150mg/kg/bw and 300mg/kgbw had a significant decrease in TG compared to high fat fed rats (negative control) ($p<0.05$; Table 4) a non-significant

decrease in TC and LDL-c and a non-significant increase in HDL-c ($p>0.05$; Table 4). Methanolic extracts dose of 150mg/kgbw and 300mg/kgbw showed a non-significant decrease in TC, TG and LDL-c compared to high fat fed rats (negative control) ($p>0.05$; Table 4).

Rats treated with dichloromethane *Solanum nigrum* (Subsp *villosum)* extracts at a dose of 150mg/kgbw and 300mg/kgbw showed a non-significant decrease in TC and a non-significant increase in TG, HDL-c and LDL-c compared to the normal control ($p>0.05$; Table 4). Furthermore, there was a non-significant difference in TC, TG, HDL-c and LDL-c in rats treated with DCM extracts dose of 150mg/kg/bw and 300mg/kgbw compared to rats treated with orlistat (positive control) ($p>0.05$; Table 4). The rats treated with extracts dose of 150mg/kg/bw and 300mg/kgbw had a significant decrease in TG compared to high fat fed rats (negative control) ($p<0.05$; Table 4) a non-significant decrease in TC and LDL-c and a non-significant increase in HDL-c ($p>0.05$; Table 4) methanolic extracts dose of 150mg/kgbw and 300mg/kgbw showed a non-significant decrease in TC, TG and LDL-c compared to high fat fed rats (negative control) ($p>0.05$; Table 4). Rats treated with dichloromethane *Solanum nigrum* (Subsp *villosum*) extracts at a dose of 150mg/kgbw and 300mg/kgbw showed a non-significant decrease in TC and a non-significant increase in TG, HDL-c and LDL-c compared to the normal control ($p>0.05$; Table 4). Furthermore, there was a non-significant difference in TC, TG, HDL-c and LDL-c in rats treated with DCM extracts dose of 150mg/kg/bw and 300mg/kgbw compared to rats treated with orlistat (positive control) ($p>0.05$; Table 4). The rats treated with extracts dose of 150mg/kg/bw and 300mg/kgbw had a significant decrease in TG compared to high fat fed rats (negative control) ($p<0.05$; Table 4) a non-significant

decrease in TC and LDL-c and a non-significant increase in HDL-c ($p>0.05$; Table 4). Methanolic extracts dose of 150mg/kgbw and 300mg/kgbw showed a non-significant decrease in TC, TG and LDL-c compared to high fat fed rats (negative control) ($p>0.05$; Table 4).

Rats treated with dichloromethane *Solanum nigrum* (Subsp *villosum)* extracts at a dose of 150mg/kgbw and 300mg/kgbw showed a non-significant decrease in TC and a non-significant increase in TG, HDL-c and LDL-c compared to the normal control ($p>0.05$; Table 4). Furthermore, there was a non-significant difference in TC, TG, HDL-c and LDL-c in rats treated with DCM extracts dose of 150mg/kg/bw and 300mg/kgbw compared to rats treated with orlistat (positive control) ($p>0.05$; Table 4). The rats treated with extracts dose of 150mg/kg/bw and 300mg/kgbw had a significant decrease in TG compared to high fat fed rats (negative control) ($p<0.05$; Table 4) a non-significant decrease in TC and LDL-c and a non-significant increase in HDL-c ($p>0.05$; Table 4, Fig 5).

Table 4: Effects of Methanol and Dichloromethane extracts of Solanum nigrum (Subsp villosum) on Lipid profiles

Treatment	TC	TG	HDL-c	LDL-c
Normal control	2.02 ± 0.04^{a}	1.29 ± 0.07^{b}	0.548 ± 0.01^{b}	0.51 ± 0.17^{b}
Positive Control	2.04 ± 0.09^{a}	1.57 ± 0.11^{b}	0.76 ± 0.03^{a}	0.48 ± 0.08^{b}
Negative Control	2.33 ± 0.04^{a}	3.13 ± 0.36^{a}	0.42 ± 0.03^{c}	0.98 ± 0.05^{a}
150mg/kgbw.MeoH	1.89 ± 0.16^{a}	2.32 ± 0.38^{ab}	0.54 ± 0.02^{b}	0.47 ± 0.15^{b}
300mg/kgbw.MeoH	1.81 ± 0.04^{a}	2.06 ± 0.21^{ab}	0.56 ± 0.02^{b}	0.46 ± 0.08^{b}
300mg/kgbw.DCM	1.84 ± 0.24^{a}	1.52 ± 0.52^{b}	0.57 ± 0.05^{b}	0.59 ± 0.11^{b}
150mg/kgbw.DCM	1.76 ± 0.11^{a}	1.64 ± 0.09^{b}	0.55 ± 0.03^{b}	0.59 ± 0.1^{b}

Mean values of Total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol(LDL-c) the different experimental groups. Bars with a similar letter are not significantly different by one-way ANOVA and Tukey's post hoc ($p>0.05$).

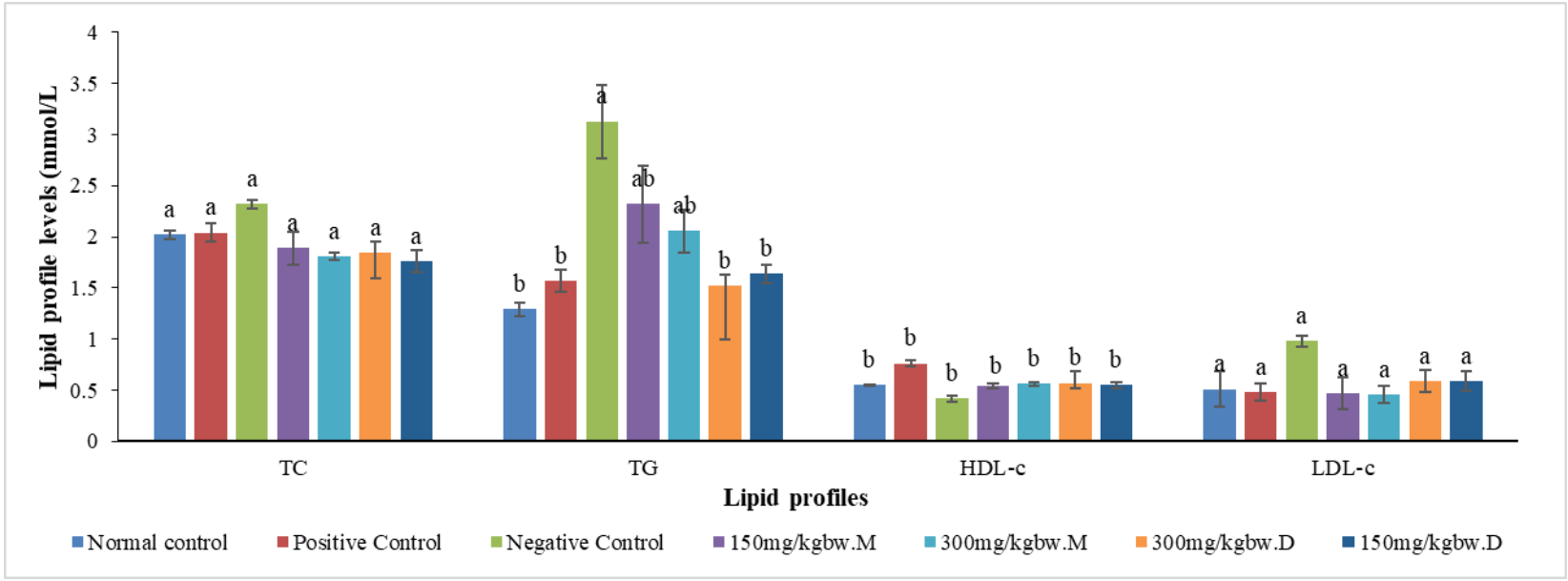


Figure 5: Mean values of TC, TG, HDL-c and LDL-c of the different experimental groups. Bars with a similar letter are not significantly different by one-way ANOVA and Tukey's post hoc ($p>0.05$).

3.4.4 Food Consumption

The results showed that the average food intake per day in a week was almost similar in all the groups except for rats administered methanolic extract dose of 150mg/kgbw, which showed a high food intake on week 6 compared to the other groups (Figure 6).

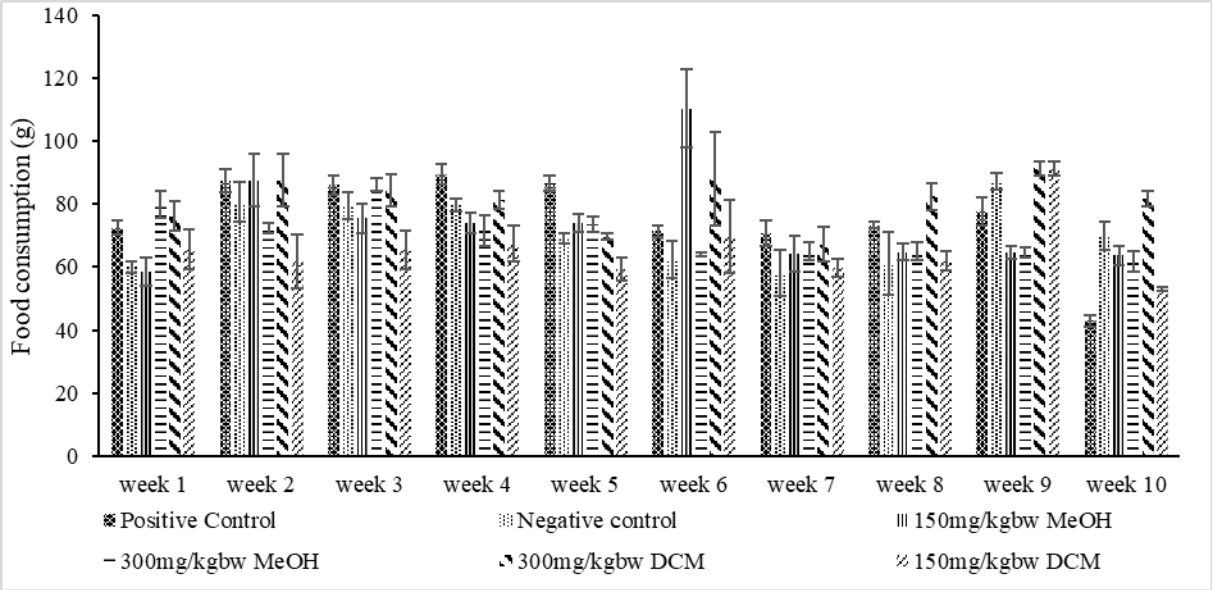


Figure 6: Food consumption rate of the different experimental groups per week

3.4.5 Adipose tissue and Liver weight

Fig 9 shows the effects of methanol and dichloromethane leaf extracts of *Solanum nigrum* (Subsp *villosum)* on adipose tissue and liver weights. There was a significance difference on adipose tissue weights and liver weight on rats administered with high fat diet compared to other treatments ($p<0.05$, Fig 7).

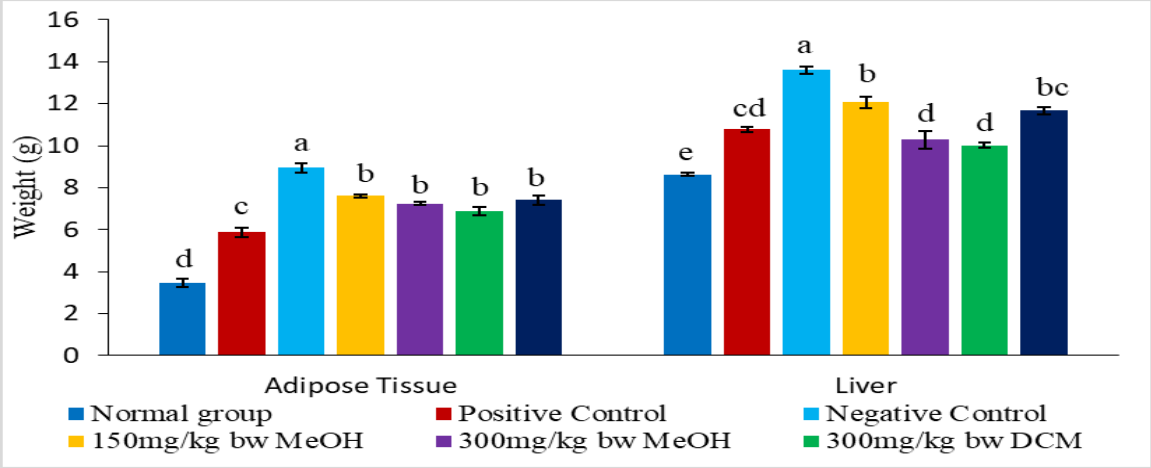


Figure 7: Mean weights of Adipose tissue and Liver of the different experimental groups. Bars with similar letter at the same tissue are not significantly different by one-way ANOVA and Tukey's post hoc ($p>0.05$).

3.4.6 Effects of Methanol and Dichloromethane leaf extracts of *Solanum nigrum* (Subsp *villosum)* on ALT, ALP and GGT of high fat fed rats

Table 5 shows the effects of methanol and dichloromethane leaf extracts of *Solanum nigrum* (Subsp *villosum)* on alanine amino transferase (ALT) alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT). The rats on high fat diet (negative control) indicated a significant increase on ALT, GGT and ALP compared to the other treatment groups.

Table 5: Effects of Methanol and Dichloromethane leaf extracts of Solanum nigrum (Subsp villosum) on ALT, ALP and GGT of high fat rats.

Treatment	ALT	ALP	GGT
Normal control	25.07±2.23^{b}	152±18.6^{b}	5.75±1.03^{a}
Positive control (orlistat 30mg/kgbw)	31.13±3.77^{b}	240±7.56^{b}	4.25±0.25^{a}
Negative control	93.9±3.8^{a}	692±191^{a}	64.8±43.2^{b}
150mg/kgbw. MeoH	34.83±6.18^{b}	224±11.7^{b}	5.5±1.50^{a}
300mg/kgbw. MeoH	13.27±1.40^{b}	195±7.53^{b}	10.75±4.27^{a}
300mg/kgbw.DCM	20.52±1.92^{b}	180±11.2^{b}	4± 0.01^{a}
150mg/kgbw.DCM	21.50±3.62^{b}	237.3±11.6^{b}	8.50±2.33^{a}

Mean values of ALT, ALP and GGT in U/L on the different experimental groups. Values with the same superscript letter are not significantly different by one-way ANOVA followed by Tukey's post hoc test ($p>0.05$).

3.4.7 Qualitative Phytochemical Composition of Methanolic and Dichloromethane Extracts of *Solanum nigrum (*Subsp *villosum)*

Qualitative phytochemical analysis indicated presence of terpenoids, tannins, saponnins, steroids, phenols, flavonoids, anthraquinones and alkaloids on both methanolic and Dichloromethane extract but did not show presence of diterpenes on the methanolic extract.

3.5 Discussion

The prevalence of several diseases is growing worldwide. Of these, obesity is one of them and has been a key problem for decades (Wilkins *et al*., 2019). A person with BMI above 30kg/m^2 is considered as obese and BMI between 25kg/m^2 – 29kg/m^2 is considered as overweight (Aoun *et al*., 2020). Excessive intake of high fat diet augmented with sucrose solution leads to obesity and other comorbidities such as diabetes, cancer, cardiovascular diseases among others (Mutiso *et al*., 2014). Several methods have been used to moderate prevalence of obesity, for example designing of drugs such as orlistat, sibutramine, semaglutide among others. However, these drugs have been found to have diverse side effects, such as constipation, heartbun, steatorrhoea, vomiting and many more. Therefore, scientist have embarked on natural medicine due to its long term effects in body weight reduction with few side effects compared to the standard drug (Tabuti *et al*., 2014).

This study was a controlled randomized design which evaluated the antiobesity effects of *Solanum nigrum* (Subsp *villosum)* on high fat diet fed rats. *Solanum nigrum* (Subsp *villosum)* (black night shade) is a medicinal / vegetable plant from the *Solanaceae* family often used by various countries. It is commonly known as Managu in Kenya and has been used globally for different treatments such as inflammation, fever and pain (Jain *et al*., 2011). *Solanum nigrum* (Subsp *villosum)* is also validated to have antitumor (Raju *et al*., 2003), anti- inflammatory (Zakaria *et al*., 2006), anti-hepatoprotective (Raju *et al*., 2003), antipyretic and anti-diabetic effects (Chauhan *et al*., 2012). The preventive and curative effects of *Solanum nigrum* (Subsp *villosum)* are associated with various phytochemical compounds present in it.

High consumption of hyper caloric diet led to significant increment in BMI compared to all other treatment groups. This study confirms that consumption of excess calorie in diet contributes to increased body weight, which leads to high body mass index compared to the normal control group. This study was similar to studies conducted on rats and mice by (Abdel-Sattar *et al*., 2018 ; Bais *et al*., 2014).

Solanum nigrum (Subsp *villosum)* leaves had anti- obesity effects on high fat fed rats as showed on this study, this was similar to studies whose report indicated plants with phytochemical compounds such as flavonoids, steroids, phenols and saponins have anti-obesity activity (Nderitu *et al*., 2017). Previous studies also report that flavonoids are most potent in suppression of obesity since they have an antioxidant effect, hence suppressing the disease through the modulation of oxidative stress in the body (Abinaya & Pavitra, 2014). According to Lunagariya *et al*., (2014) they indicated that flavonoids inhibit the pancreatic lipase enzyme by inhibiting dietary fat absorption and increasing excreation of fats in faeces, hence this study postulates that reduction of body mass index was due to that effect.

The serum levels of low-density lipoprotein-cholesterol, total cholesterol and triglycerides were higher on the high fat fed diet group compared to the normal controls. This was in agreement with studies conducted by El-Shiekh *et al*., (2019); Moon *et al.*, (2013) on rats and humans. Although the rats were hyperlipidemic, rats administered with the *Solanum nigrum* (Subsp *villosum)* extracts were hypolipidemic. This was similar to a study conducted by Bais *et al*., (2014) whose study showed supplementation of *Moringa oleifera* led to increase in HDL and reduction of LDL, TC and TG on high fat fed rats.

Previous reports showed presence of bioactive compounds account for the observed bioactivity for example; presence of phytosterols reduced Triacylglycerides and cholesterol by down regulating expression of lipogenic genes (SREBP1 and FAS) and proteins involved in cholesterol biosynthesis such as; sterol regulatory element binding transcription factor -2 (SREBP- 2) and 3-hydroxyl 3-methyl glutaryl coA reductase (HMGCR) (Chavez-Santoscoy *et al*., 2014). Studies conducted by (Gylling & Simonen, 2015) also indicated that saponins present in the extracts inhibit cholesterol micellization by interfering with the intestinal solubility, hence increasing high density lipoproteins while phytosterols lower low density lipoprotein. This report was similar to our study, and hence it can be concluded that *Solanum nigrum* (Subsp *villosum)* had the same therapeutic effect.

The liver enzymes are markers that indicate the state of the liver. A high fat diet damages the liver leading to the production of excess alkaline phosphatase, aspartate aminotransferase, and gamma glutamyl transferase. This study showed an increase in ALP, AST and GGT among the high fat diet group compared to the normal controls and other treatment groups similar to a study done by Kim *et al.,* (2018) on mice. According to previous studies, this occurs due to abnormal glycosylation, which is associated with changes in hepatocytes and hepatosteatosis which leads to lipid storage in the cytoplasm thus increasing serum levels of AST, ALP and GGT (Kim *et al*., 2018). However, the treated groups had lower ALT, ALP and GGT levels comparable to that of high fat diet group. Hence, we postulated that *Solanum nigrum* (Subsp *villosum)* is safe for consumption similar to a study done by Moradi *et al*., (2019).

There was a significant increase in glucose level on high fat fed rats at week 10 compared to other treatment groups. However, the decrease in serum glucose on other groups was associated with glucose sensisitivity. Therefore, we hypothesize that *Solanum nigrum* (Subsp *villosum),* like Luo & Liu, (2016) and Wang *et al*., (2016) experiments on high-fat-fed mice, was able to reduce insulin resistance in the high-fat-fed rats.

Food intake was almost similar across the groups and similar to other studies which indicated food intake is not dependent on body weight. For example, a study by Luo *et al.*, 2016 reported the reduction in weight as parallel to food intake. He stated that reduction in body mass index could be as a result of energy utilization and not necessarily energy consumed. A study conducted by (Patra *et al*., 2015) reported that alkaloids reduce food intake by suppressing appetite. However, this study showed no similarities to the study.

There was a significant reduction in weight among the treated groups compared to the high fat fed rats similar to a study conducted by Ji *et al*., (2017a). The increase in liver and adipose tissue was as a result of accumulation of triglycerides (Luo & Liu, 2016). This study hypothesizes that the reduction in weight of the adipose tissue was due to the inhibitory effects of the extract, which occurred through the inhibition of the differentiation of 3T3-L1 predadipocytes cells to adipocytes by down regulation expression of PPARγ (Guo *et al*., 2021). Adipogenesis was inhibited through the suppression of the adipogenic specific proteins in 3T3-L1 cells (Ji *et al*., 2017b).

This study concludes that *Solanum nigrum* (Subsp *villosum)* reduces BMI, has hypolipidemic effect and possess anti-obesity phytochemicals with no adverse effects on

liver function. Therefore, recommends consumption of *Solanum nigrum* (Subsp *villosum)* as a supplement used to prevent obesity.

CHAPTER FOUR

MORPHOLOGICAL AND MOLECULAR IDENTIFICATION of *Solanum* specie

Abstract

Solanaceae is a taxonomic family of important angiosperms with high commercial and economic value and is mostly used as food, medicine or as a spice. Taxonomic assignment based solely on morphological markers within this family has been shown to give unreliable, inaccurate and contradictory results due to phenotypic plasticity and ontogenetic contingency. Morphological traits showing similarities include; shape and texture of the leaf, fruit color, size of the leaf among others. In this study we used ITS2 and rbcl as DNA barcodes to assign the species taxonomic rank to a member of *Solanaceae* that showed medicinal value against obesity, since it reduced body mass index, glucose levels and cholesterol levels on high-fat-fed rats.

Methodology: Fresh *Solanaceae* leaves were collected from Limuru sub county, Kiambu County and transported to the department of biogical sciences for morphological identification and authentication. Voucher specimen was deposited at the herbarium while the rest was shipped to the molecular laboratory for DNA extraction. DNA was extracted from the leaves of *Solanaceae* a plant (*Kat_001*) that showed anti-obesity effects on rat models. PCR was conducted using the primers ITS2 and rbcl at an annealing temperature of 58^{o} C for both primers and extension at 72^{o} C for 35 cycles. Phylogenetic inference was based on ITS2 and rbcl and multi-locus inference based on a concatenation of both ITS2 and rbcl using maximum likelihood (ML) and maximum parsimony (MP) algorithms.

Results: Concatenation of the different sequences used to infer phylogeny in the ITS and rbcl was successful and indicated that *Kat_001* is *Solanum nigrum* (Subsp *villosum).*

Conclusion: The agreement between ITS2 and RBCL sequences, and the combined set of rbcl and ITS2 sequences, together with morphological data strongly suggests *Kat_001 is Solanum nigrum (*Subsp *villosum)* and is from the larger *Solanum nigrum* complex.

4.1 Introduction

Solanum nigrum L is a dicot from the family *Solanaceae*, known as black night shade in English, Makoi in india and Managu in Kenya (Saleem *et al*., 2009; S. Sharma & Dogra, 2018). It shows genetic diversity since it's a complex of many species such as *S. scarbum, S. americanum, S. nigrum, S. physalifolium and S. villosum* (Hameed *et al.*, 2017). *Solanum nigrum* is distributed in various regions of the globe from tropical to temperate and from high altitudes to low altitudes. It grows up to 1.3 m high with high-spreading branches (Ronoh *et al*., 2018). Others have small leaves while other species contain broad leaves that are either serrated, oval shaped and green in colour. They are differentiated based on their berries some have purple to black while others have green to orange fruits (Janepher *et al*., 2021). In Europe, *Solanum nigrum* is considered as a bothersome and toxic weed in agriculture and in open fields and therefore it is not used for either consumption or medicinal value, while in the indian communities various studies show that *Solanum nigrum* is used for consumption due to its medicinal properties (Nyeem *et al*., 2017). Traditionally, *Solanum nigrum* has been used to cure conditions like hepatitis, asthma, diarrhea, ulcers, fever, and stomach issues (Miraj, 2016). For instance, in Telangana region *Solanum nigrum* plant was known as peddakasha pandla koora and was mainly used in the treatment of tuberculosis. In Kage soppu in Karnataka and Tamil Nadu the plant is known as manathakkali keerai and is used to treat mouth ulcers during winter periods and is also used as a vegetable (Nyeem *et al*., 2017). Previous studies indicated that boiled berries and leaves of the plant were used to alleviate liver-related ailments such as jaundice. On the other hand, root juice was used

against whooping cough and asthma (Teklehaimanot *et al*., 2015). *S. nigrum* is a widely used plant in oriental medicine where it is considered to be antipyretic, antitumorigenic, antioxidant, hepatoprotective, anti-inflammatory and diuretic. A few experiments confirmed that the *Solanaceae* plant inhibits the growth of cervical carcinoma (Nyeem *et al*., 2017). In Africa, different ethnicities consider *Solanum nigrum* as a vegetable and has also been used for different treatments (Potawale *et al*., 2008).

The vegetative features of *Solanum* specie show diverse phenotypic variations in their stem, leaf shape, size and plant habit. Few studies indicate that *Solanum nigrum* spread to Africa from Eurasia (Matasyoh & Bosire, 2016).

In Kenya, *Solanum nigrum* is also known as managu and usually grow as a weed in moist habitat, and in different types of soils such as stony, dry, shallow and can be cultivated both in subtropical and tropical climatic regions (Wesonga *et al*., 2016). In Kenya, the unripe berries are squeezed on infants' gums to ease pain in the teething stage, treat stomach aches, sooth tooth ache, treat tonsillitis, malaria, colds, coughs, diabetes, high blood pressure and chest pain (Kimiywe *et al*., 2007).

In addition, hybridization of *Solanaceae* specie affects global morphological characteristics, for example, F1 hybrids appear different from F2 progeny (Oyelana & Ugborogho, 2008). This observation makes it difficult to accurately assign taxonomic ranks to *Solanaceae* species using morphological data. This is made more complex by the fact that *Solanum* genus is species complex (Knapp *et al*., 2013; Mohy-Ud-Din *et al*., 2010). Additionally, DNA barcoding has thus become a gold standard in identifying species. The use of short universal standardized DNA sequences as DNA barcodes has

proven to be an accurate method of identifying medicinal plants (Saddhe & Kumar, 2018; Yu *et al*., 2021). The use of more than one DNA barcode is the current method for bridging limitations arising from nuclear and organelle molecular data (Liu *et al*., 2017). Literature diversity on Solanaceae has increased rapidly over the years and hence this study aimed at identifying *Solanaceae* specie, by use of ITS2 and rbcL DNA barcodes (Gebhardt, 2016).

4.1.1 Ribulose 1,5- biphosphate carboxylase/ oxygenase (rbcl) and internal transcribed spacer

Solanum nigrum Linn contains over 2000-3000 species (Jabamalairaj *et al*., 2019), in this study one specie was harvested and used to test for anti-obesity effect on high fat fed Sprague dawley rats. Although, morphological identification had been done by a taxonomists, it is not an accurate method of identifying the different related species since their phenotypic characteristics are almost similar (Yang *et al*., 2019). Hence, we considered molecular identification using two universal plant primers which included; internal transcribed spacer regions (ITS2) and ribulose biphosphate carboxylase (RBCL). DNA barcoding is a tool used to discriminate species by identifying and documenting plant diversity in certain taxonomic groups. This is done through amplification of the DNA barcode region among large assemblies of taxa hence providing species discrimination (Kusai *et al*., 2016; Osathanunkul *et al*., 2018). Nuclear-encoded ribosomal internal transcribed spacer regions (ITS2) and large subunits of Rubisco (rbcl) were adopted as standards. This study employed the two DNA barcodes ITS2 and RBCL to determine the species and the highest performance marker.

4.2 Methodology

4.2.1 Morphological identification of the plant

The leaves were identified by a taxonomist from the biological sciences department, and among the characteristics evaluated were leaf shape, leaf margin, fruit color, and leaf surface. In order to preserve the quality of the DNA before extraction, the leaves were subsequently delivered to the molecular biology lab at the Department of Biochemistry covered in foil and kept there overnight at -20 C.

4.2.2 DNA extraction from *Solanum nigrum* leaves

Thirty (30) mg of leaves were used to extract genomic DNA using the cetyl trimethylammonium bromide (CTAB) technique. The leaves were homogenized using motor and pestle into a fine paste in 500 µl CTAB buffer and later transferred into a polypropylene tube where it was mixed thoroughly using a votex. After 30 minutes in a 65°C water bath, the tube was centrifuged for 5 minutes at 14000 x g. A fresh tube was created, the supernatant was placed in, and 5 µl of RNAse solution was added. The tube was then incubated at 37 °C for 20 minutes. An equal volume of phenol/chloroform was added to the solution at a ratio of 24:1 and later vortexed for 5 seconds then centrifuged for 1 minute at 14000 x g in order to separate the phases. In order to precipitate the DNA, the supernatant was transferred into a fresh tube, 0.7 µl of cold isopropanol was added, and the mixture was incubated at -20 °C for 15 minutes. After that, the sample was centrifuged for 10 minutes at 14000 x g. Afterwards, the supernatant was removed without touching the particle and rinsed with 500µl of ice-cold 70% ethanol. The ethanol was then decanted and the pellet dried long enough in order to remove the ethanol completely. DNA concentration was evaluated spectrophometrically using Nanodrop^{TM} 2000/2000c spectrophotometer at 260 nm and the purity determined by considering the

ratios of OD 260 nm /280 nm and OD 260 nm /230 nm. Further quality of DNA was confirmed through agarose gel electrophoresis as follows: Agarose gel electrophoresis was prepared by weighing 0.8% of agarose powder. The powder was poured into a conical flask containing 100 ml of 1 x TBE buffer and then heated to boil in a microwave for 3 minutes. The conical flask was then removed and allowed to cool for few minutes, later 1 µl of Ethidium bromide was added to the gel and stirred in order to mix evenly. Gel combs were arranged in a tray on a flat surface area for creation of wells for loading the DNA sample. The gel was allowed to dry at room temperature for a few minutes and thereafter shifted to a gel electrophoresis tank (amplisize; Bio rad Laboratories GelRed Nucleic Acid) containing 1x TAE buffer for 1 hour at 80 volts and visualized by UV transillumination. O' geneRuler 1 kb DNA ladder, 0.1µg/µL (Thermo Fisher Scientific, California, USA) was used as a marker for genomic DNA sizes. A mix of Plain dye together with the master mix was used as the negative control. The DNA pellet was later dissolved in 20 µl TE buffer and stored at -20 ˚C awaiting PCR assay.

4.2.3 Amplification

Polymerase chain reaction was performed using 0.4 µM of ITS2 and RBCL primers (see table 6) along with PCR Master Mix (one Taq quick load 2X Master Mix with standard buffer M0486S) and 1µl of DNA with a concentration of >50ng/ µL. The detection mix for each gene contained 9.5 µl of nuclease free water, 1µl of each primer, 12.5µl one Taq quick load 2x master mix with standard buffer and 2µl of template genomic DNA. The total volume was 25µl reaction. Amplification was done under the following thermo cycling conditions: Initial denaturation at 95 ˚C for 30 seconds and 35 cycles of amplification, 1 minute at 58 ˚C for annealing, 5 minutes at 68 ˚C for final extension. This

was performed using applied biosystem 96 well veriti Thermal cycler (ThermoFischer Scientific, USA). The PCR amplicons were separated by gel electrophoresis at 80 volts for 1 hour on 2% agarose gel (Amplisize, Bio-Rad Laboratories) using 1 x TAE Buffer containing GelRed Nucleic Acid and visualized under UV transillumination. 1 kb DNA ladder of 0.1µg/µL (Thermo Fisher Scientific California, USA) was used as a marker for the amplicon gene sizes.

Table 6: Primers used for DNA barcoding

Primer Name	Annealing temperature	Direction	Sequence (5'-3')	References
Rbcl	58°C	Forward Reverse	CTGTATGGACCGATGGACTTAC CGGTGGATGTGAAGAAGTAGAC	(Nderitu *et al.*, 2023)
ITS2	58°C	Forward Reverse	GAAGGAGAAGTCGTAACAAGG TCCTCCGCTTATTGATATGC	(Nderitu *et al.*, 2023)

4.2.4 Sequencing of amplicons / sequence alignment and phylogenetic analysis

The PCR amplicons were cleaned and sequenced at the University of Nairobi Institute of Tropical Infectious Diseases (UNITID) using Sanger sequencing method. The resultant chromatograms were visually inspected and manually cleaned within the BioEdit software version 7.2.5 (Hall, 1999) (BioEdit). Since the sequencing for each sample was done both in the forward and the reverse directions, two chromatograms were obtained for each sample. For each individual chromatogram, the noisy regions located at the beginning and at the end of the chromatogram were deleted. The sequence was then visually investigated for any miscellaneous bases and for any that were found, the chromatogram peaks were employed to determine the most likely base to replace the miscellaneous base. The cleaned sequence was then saved in FASTA file format. This

was carried out for all of the sequences. Each pair of sequences that were sequenced using the same pair of primers were transferred to BioEdit for contiguous sequence (contig) creation. The resultant consensus sequences were saved as FASTA files and used in downstream analysis.

A BLASTN search was carried out for each contig. All the parameters and settings in the BLASTN algorithm were left in their defaults except for the following: uncultured and environmental samples were excluded from the search and the BLASTN algorithm was optimized to display highly similar sequences. Suitable sequences from BLAST were selected for analysis. A suitable sequence had to satisfy these criteria: (1) the percentage identity had to be at least 98%, (2) the sequence had to have a query coverage of at least 90% and (3) the expected value had to be zero.

The selected sequences corresponding to each contig, together with each contig were transferred to the Molecular Evolutionary Genetic Analysis software version 11.0.10 MEGA11 (Tamura *et al*., 2021). Within MEGA11 a multiple sequence alignment (MSA) was carried out using the algorithm MUSCLE with all settings and parameters left in their default (Edgar, 2004). Once the alignment was done, it was visually inspected to remove erroneous sequences, correct any errors such as unusual gaps, base insertion among others. The MSA was then carried out once more after correcting the sequences, and the updated alignment was visually evaluated once more to see whether any sequences needed to be eliminated. Once the correct MSA was determined a final MSA was carried out.

The MSA was then subjected to model selection within MEGA11 to determine the best nucleotide substitution model. The model with the smallest Bayesian Information

Criterion was selected as the best substitution model, and was used in the construction of neighbour joining trees (NJ) within the MEGA 11 environment. Construction of maximum parsimony trees (MP) did not require a model and thus was determined using the default settings of MEGA 11. On the other hand, construction of maximum likelihood trees (ML) was done using the 'one click Mode' algorithm in the phylogeny (ML). The phylogeny of MP, ML and NJ trees were tested using bootstrapping with 1000 bootstraps for each method with the substitution model added (Yang & Rannala, 2012). Phylogeny was inferred using phyML and the tree rendered by TreeDYN (Chevenet *et al*., 2006; Guindon *et al*., 2010).The resulting phylograms were saved in NEWIK format and edited within MEGA11.

4.3 Results

The examined morphological features of the *Solanum* genus used showed the following characteristics (See table 7).

Table 7: Assessed morphological features of Solanum genus

	Trait	**Features**
1.	Leaf shape	Ovate
2.	Leaf margin	Sinuate
3.	Fruit color	Green to orange
4.	Leaf surface	Hairly

4.3.1 Phylogenetic Analysis

In this study universal primers ITS2 and rbcl were used as the DNA barcodes for *Solanaceae*. For the three data sets produced by MSA, phylogeny was inferred using ML, MP, and NJ methods. With reference to each dataset, the topologies of all the trees generally agreed with one another. A notable exception was observed with the phylogeny that was inferred using ITS2 dataset. While both the MP (figure 12) and ML (figure 11)

trees showed that *Kat_001* clustered with *S. villosum*, the NJ tree (figure 13) clustered the *Kat_001* with two members of *S. physalifolium* with a bootstrap value of 0.7. This clade was paraphyletic to the clade that housed *S. villosum*, this *S. physalifolium- villosum* clade was supported by a relatively weak bootstrap value of 0.67. In contrast, the clustering of *Kat_001* with *S. villosum* observed within MP and ML had stronger bootstrap support: 0.81 and 0.86 respectively. It was still observed that *S. physalifolium* and *S. villosum* formed a monophyletic clade within both MP and ML but supported by 96% and 91% of bootstraps respectively.

The sequences that derived from rbcl were also used to construct ML (figure 14), MP (figure 15) and NJ (figure 16) phylogenetic trees. All the trees placed Kat_001 with members of *S. nigrum* with varying bootstrap supports. The highest bootstrap value was with ML then MP and NJ with 0.8, 0.77 and 0.59. It was not possible to delimit the specific sample that rbcl clustered with within the ML phylogeny, the entire clade that harbored *Kat_001* was polytomy, and thus unresolved. The NJ tree showed that *Kat_001* formed a monophyletic clade with MT782383 from GenBank that was a member of *S. nigrum.* In the MP tree *Kat_001* was ancestral to a monophyletic clade consisting of MT782383 and KU556630 (figure 15).

The aligned sequences of rbcl and ITS data were concatenated to produce a single dataset that was used to construct MP, ML and NJ trees. With a bootstrap value of 0.89, *Kat_001* clustered with four *S. nigrum* sequences. This was replicated within MP with a bootstrap value of 0.74 (figure 9) and NJ (figure 10) with a bootstrap value of 0.65. The ML (figure 8) and MP trees showed that the sequences that were obtained from GenBank clustered into three distinct clades. The tree constructed using the NJ method showed a radically

different topology with clustering into two clusters. However, this was not necessarily a disadvantage of inferring phylogeny via NJ, the concatenated ML and MP trees were able to resolve distinct species boundaries for 11 taxa each. Cluster I of the ML tree had 7 clades with clear clustering into species, cluster II had 1 and cluster III had 3 clusters of species in monophyletic clades. The distribution in the concatenated MP tree for cluster I, II and III was 6, 1 and 4 species delimited clades respectively. The two clades observed in the NJ tree had 8 species delimited in the first cluster and 2 in the second. The investigation of the topology of NJ revealed that the designated clusters I and II in the ML and MP trees were pooled together to form cluster I in the NJ tree. *S. villosum* did not form a monophyletic clade in the NJ tree thus explaining the absence of a single species delimitation compared to MP and ML.

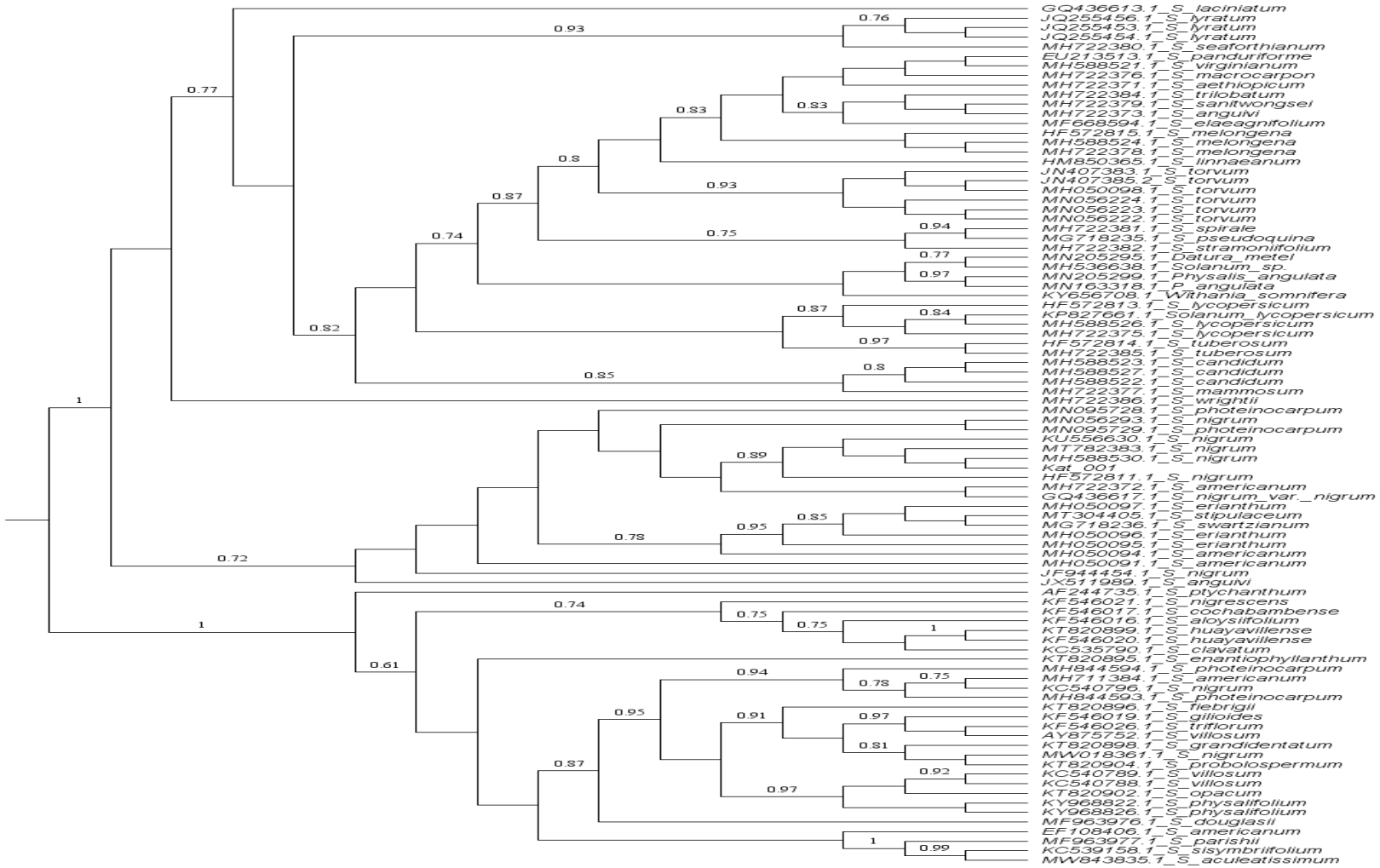


Figure 8: A rooted ML tree inferred from concatenated ITS2 and rbcl data sets. The values are proportion of bootstrap values.

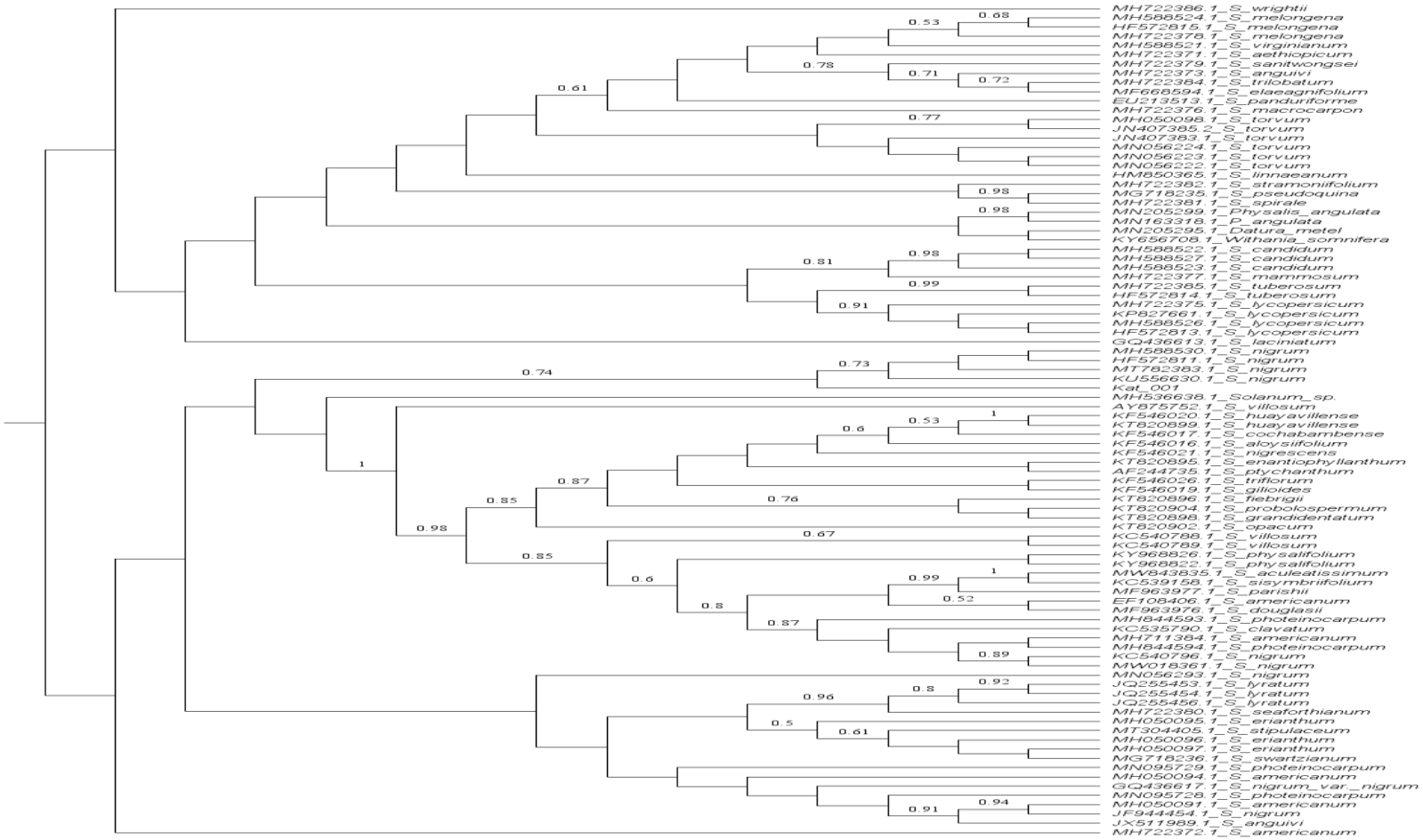


Figure 9: A rooted MP tree inferred from concatenated ITS2 and data sets. The values are propotion of bootstrap values from 1000 replicates, only higher than 0.5 are shown.

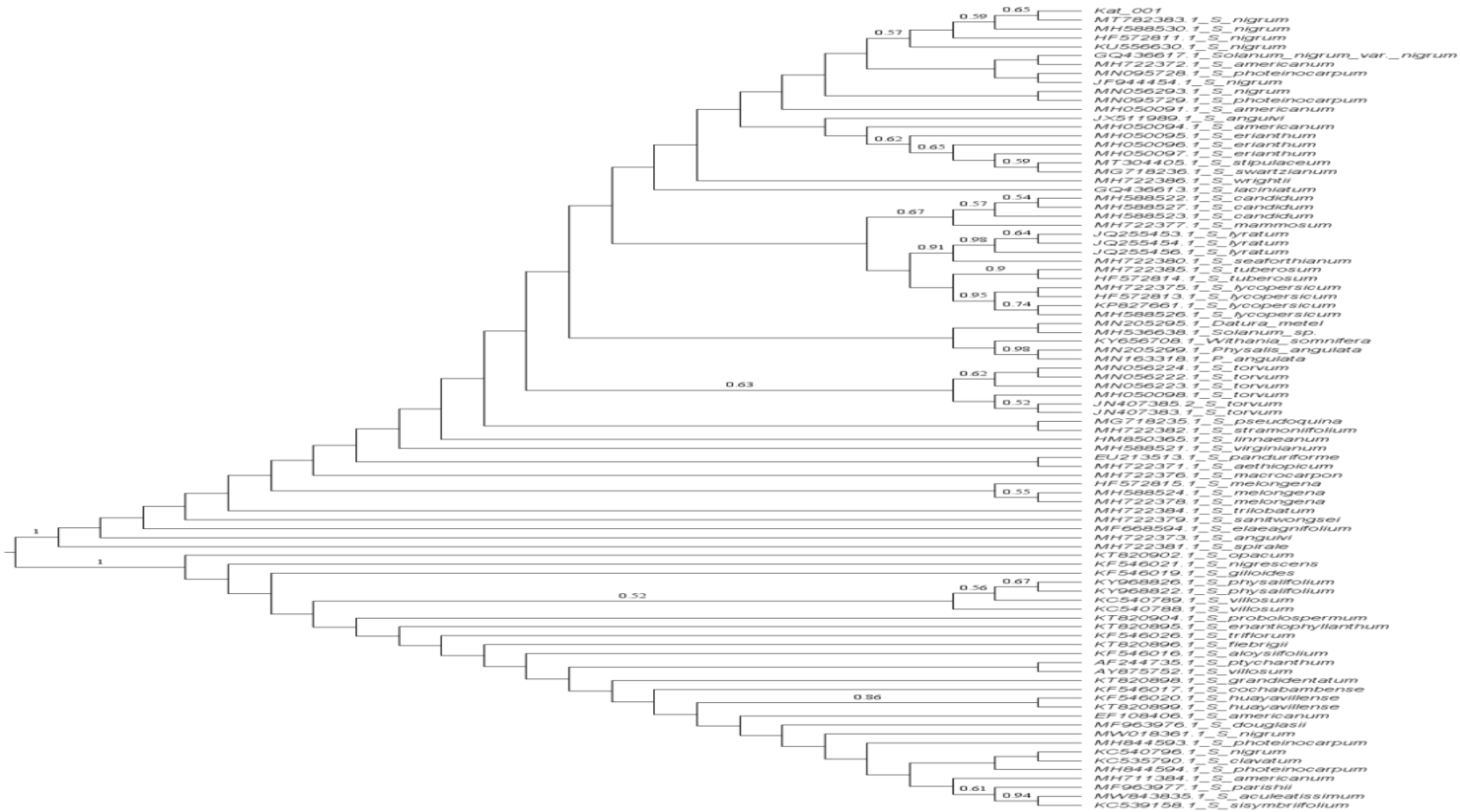


Figure 10: A rooted NJ tree inferred from concatenated ITS2 and rbcl data sets. The values are proportion of bootstrap values from 1000 replicates, only higher than 0.5 are shown.

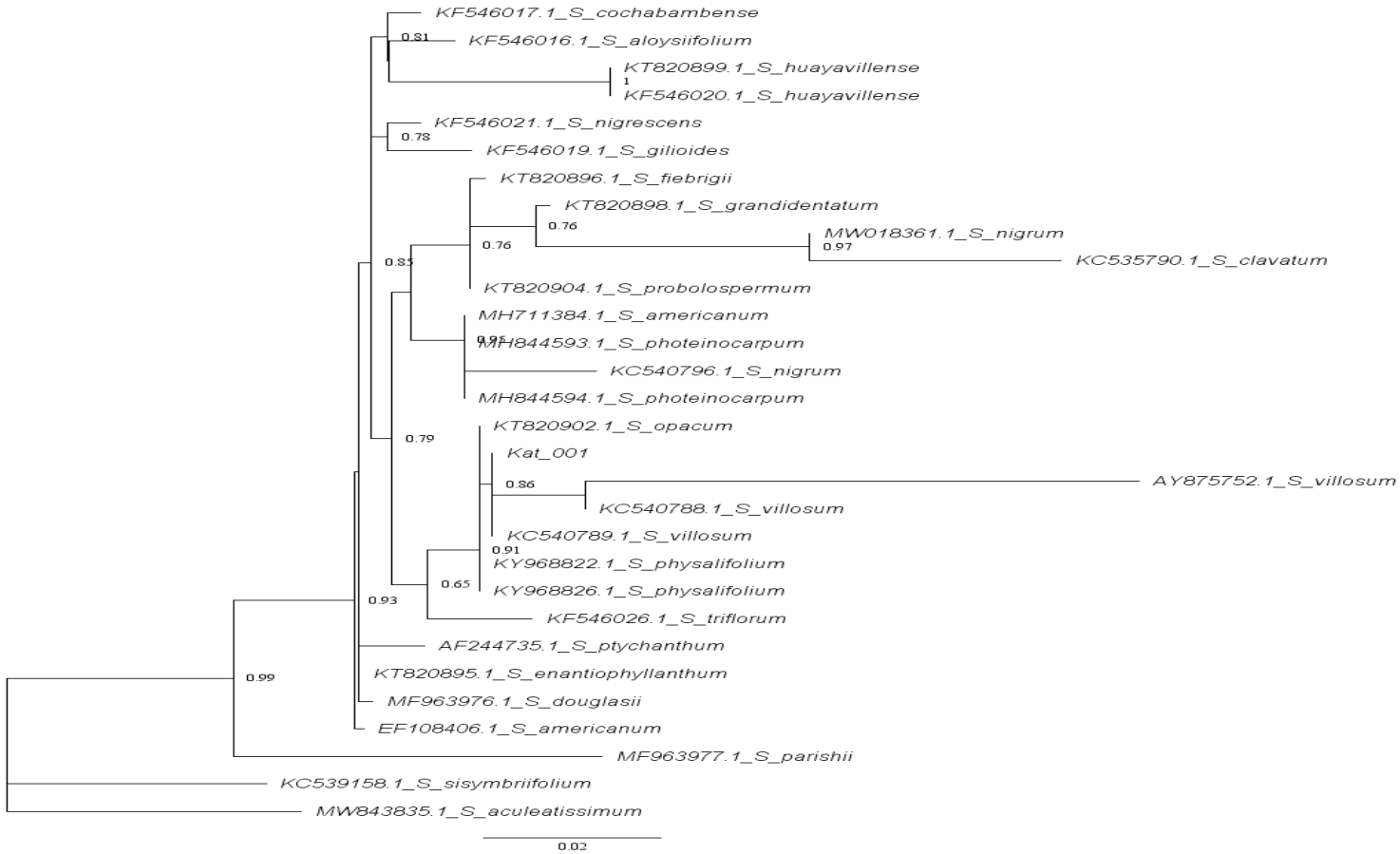


Figure 11: A rooted ML tree inferred from ITS2. The values are proportion of bootstrap values from 1000 replicates, only higher than 0.5 are shown.

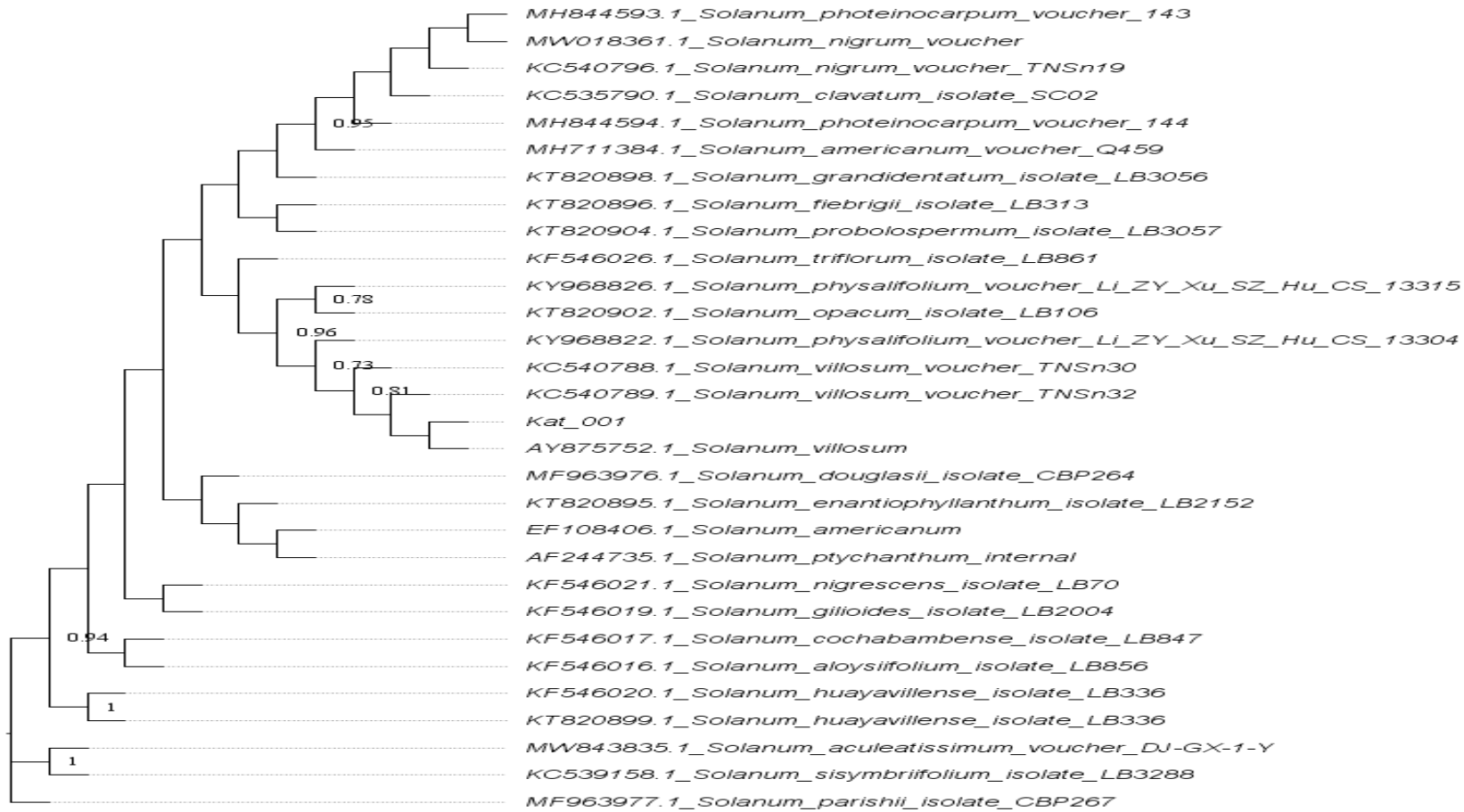


Figure 12: A rooted MP tree inferred from ITS2. The values are proportion of bootstrap values from 1000 replicates, only higher than 0.5 are shown.

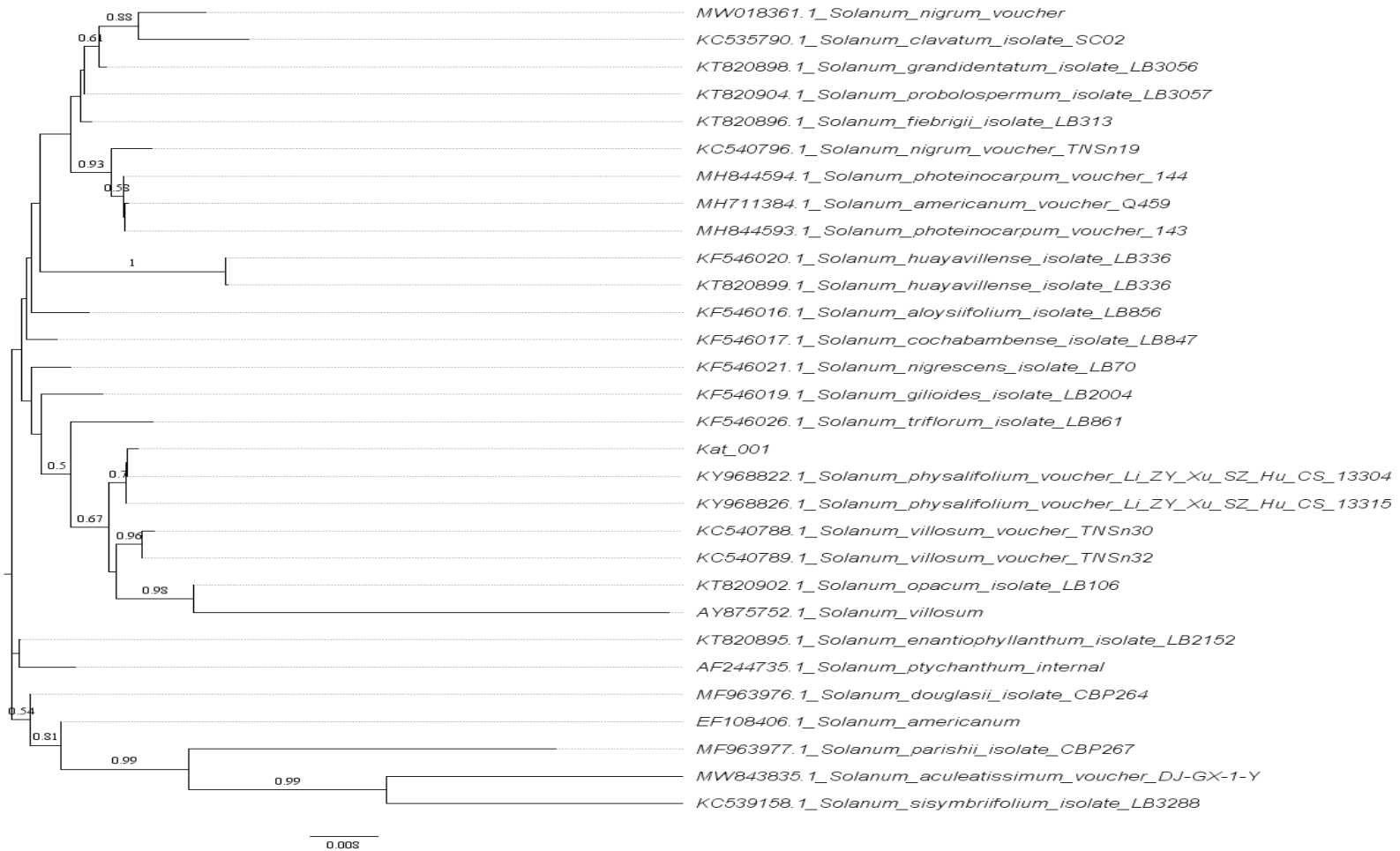


Figure 13: A rooted NJ tree inferred from ITS2. The values are proportion of bootstrap values from 1000replicates,only higher than 0.5 are shown

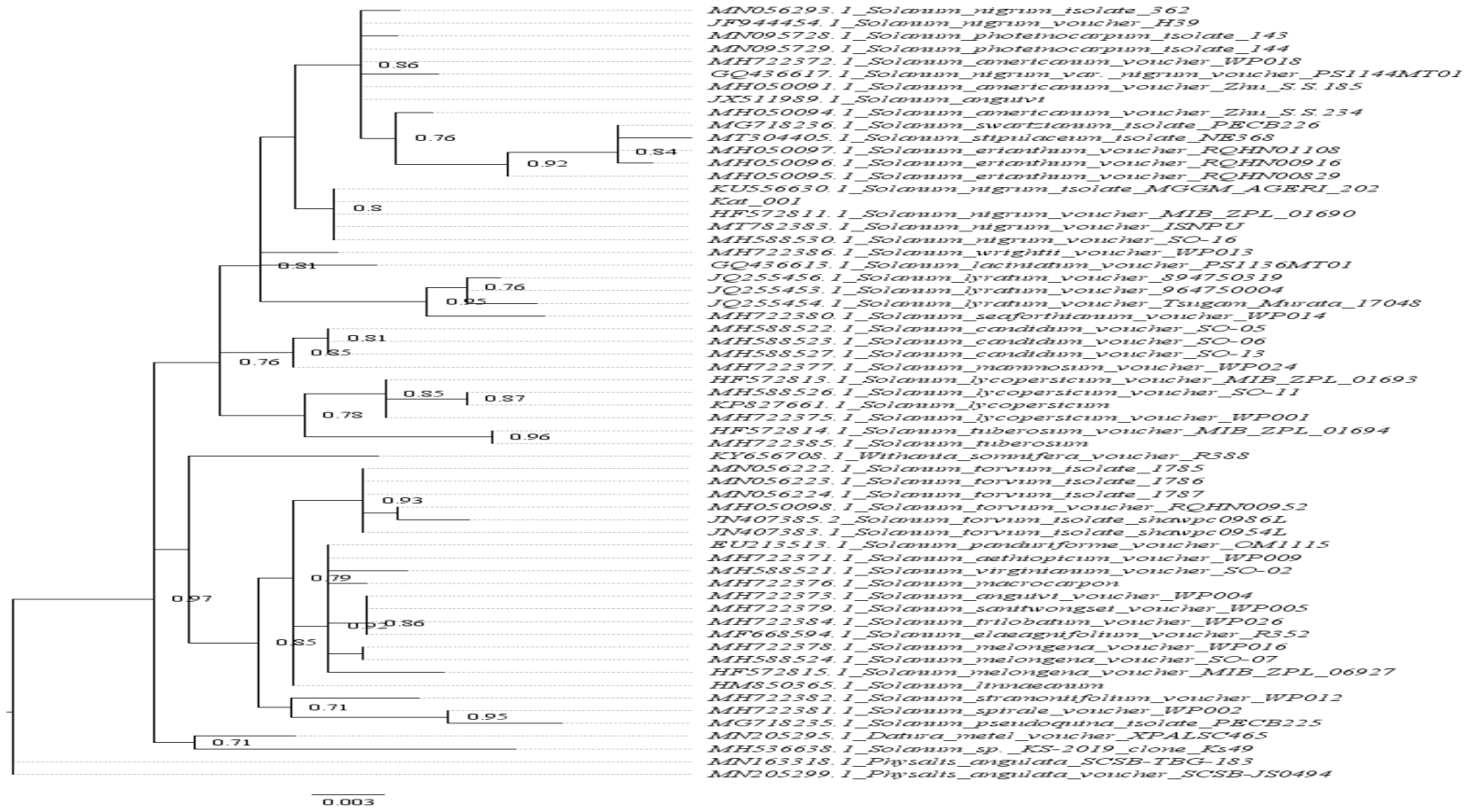


Figure 14: A rooted ML tree inferred from RBCL. The values are proportion of bootstrap values from 1000 replicates, only higher than 0.5 are shown.

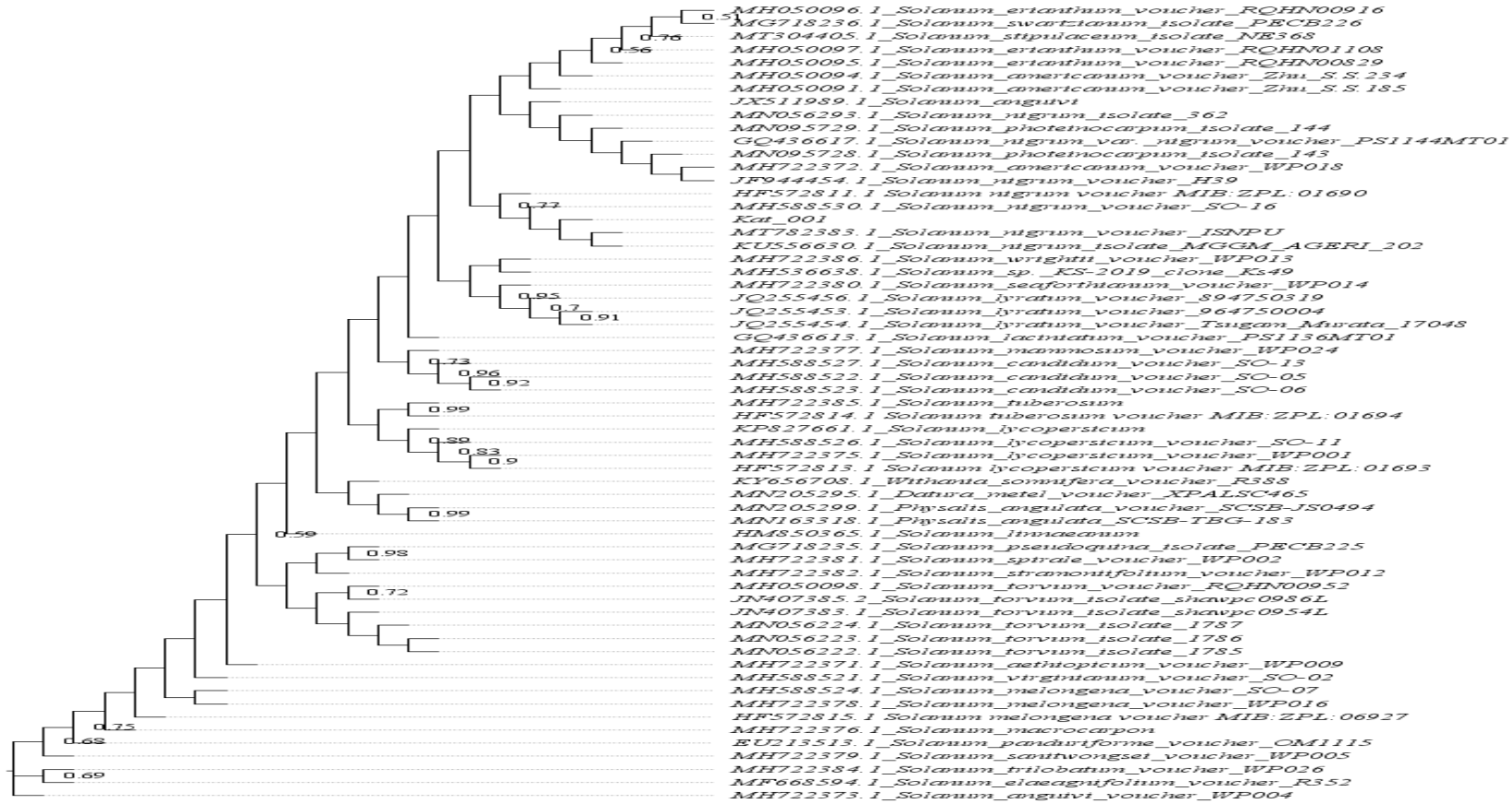


Figure 15: A rooted MP tree inferred from RBCL. The values are proportion of bootstrap values from 1000 replicates, only higher than 0.5 are shown

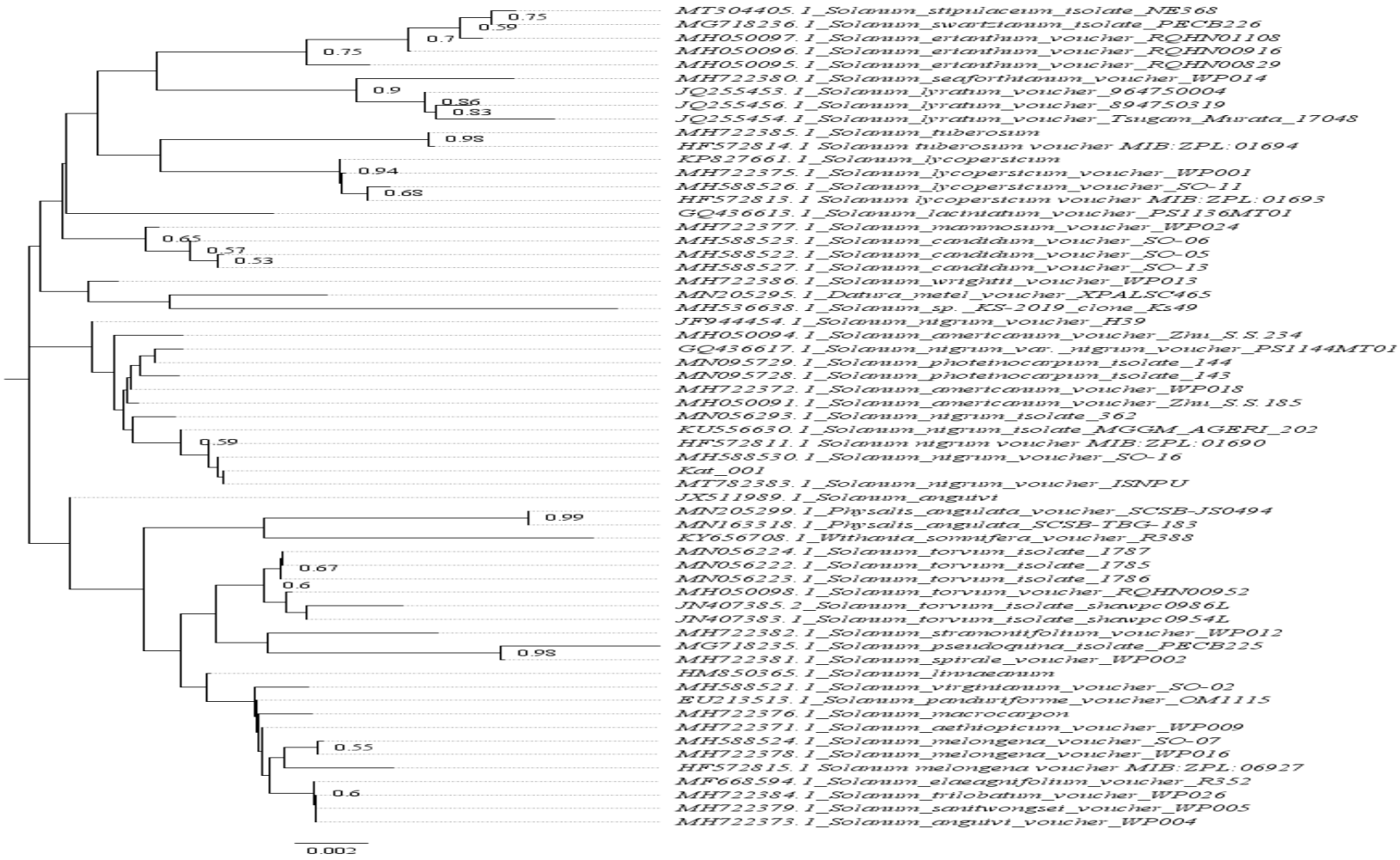


Figure 16: A rooted NJ tree infered from RBCL. The values are proportion of bootstrap values from 1000 replicates, only higher than 0.5 are shown

4.4 Nucleotide composition

To assess the nucleotide composition of the ITS sequence and rbcL sequences, GenBank sequences used in phylogenetic reconstruction belonged to the same taxon as the sample and were used to calculate the composition of the bases of such taxon. The sample ITS1_rc had the longest nucleotide length of 698 nucleotides followed by KC540789.1 at 690 nt then finally KC540791.1 at 687 nt. Cytosine had the highest frequency with an average of 216.33 nt which translated to 31.23% of all the nucleotides. This was followed by Guanine which had 30.13% of the total nucleotides. With this high frequency in guanine and cytosine, the G+C content was also high at an average of 61.40%. ITS1_rc had the highest G+C content of 61.46% (see table 8). In terms of adenine (A) and thymine (T) composition, adenine had the highest percent of 19.93 while thymine (T) was 18.70 percent.

Table 8: Nucleotide composition of ITSI_rc

Isolate	**Length**	**Adenine**		**Cytosine**		**Guanine**		**Thymine**		**G – C content %**
		freq	***%***	***Freq***	***%***	***freq***	***%***	***freq***	***%***	
KC540789.1	690	137	19.9	217	31.4	206	29.9	130	18.8	61.3
KC540791.1	687	136	19.8	216	31.4	206	30	129	18.8	61.43
ITS1 rc	698	140	20.1	216	30.9	213	30.5	129	18.5	61.46
Mean	691.67	137.67	19.93	216.33	31.23	208.33	30.13	129.33	18.70	61.40
SD	5.69	2.08	0.15	0.58	0.29	4.04	0.32	0.58	0.17	0.09

A similar assessment was conducted with the partially amplified and sequenced rbcL gene. Three sequences belonging to *S. nigrum* together with RBCL2_rc were obtained from gen bank for analysis. The average length of the sequence was 706.5 nt with rbcL2_rc having the longest length of 714 nt with the least being MH588530. Unlike, ITS sequences, Thymine had the highest frequency with an average of 205.5 nt which translated to 29.1% this was followed by adenine with 202.0 nucleotides on average which computes to 28.6% with this, the A+T content was thus higher than the G+C content which an average of guanine at 22.7% and cytosine at an average of 19.6% totaling to overall GC content average of 42.3%. Except for the nucleotide length, all the other parameters in the analysis had standard variations of less than 3, this means that these compositions are highly conserved and do not vary much within the species taxon (See table 9).

Table 9: Nucleotide composition of RBCL2_rc

Isolate	**Length**	**Adenine**		**Cytosine**		**Guanine**		**Thymine**		**G – C content %**
		Freq	***%***	***freq***	***%***	***freq***	***%***	***freq***	***%***	
MH588530	701	198	28.2	139	19.8	161	23	203	29	42.8
GQ436617	704	203	28.8	136	19.3	159	22.6	206	29.3	41.9
MN056293	707	202	28.6	138	19.5	159	22.5	206	29.1	42.08
RBCL2 rc	714	205	28.7	140	19.6	162	22.7	207	29	42.3
Mean	706.5	202.0	28.6	138.3	19.6	160.3	22.7	205.5	29.1	42.3
SD	5.57	2.94	0.26	1.71	0.21	1.50	0.22	1.73	0.14	0.39

4.5 Discussion

Members of the *Solanaceae* family usually form species complexes. The cryptic nature of members of these complexes makes it very difficult to assign taxonomic ranks to these members using morphological data alone (Chiarini *et al*., 2018). Recently several authors have preferred multiphasic approaches to determine species boundaries for several cryptic species (Savi *et al*., 2019). One of the more common approaches have been complementing morphological data with one or more molecular data, this was the approach that was used in the current study.

The morphological data placed the sample used in the current study in the genus *Solanum* sp. but it was not possible to assign a lower taxonomic rank to the sample. Based on the suggestion of (Gao *et al*., 2010) the primer ITS2 was used for DNA barcoding to supplement this morphological data. The phylogenetic trees constructed based on ITS2 sequences were incongruent with regard to the clustering of the *Kat_001*. Both the ML and MP trees agreed that *Kat_001* was possibly a member of *S. villosum*. The ML tree suggested that the sequence KC540789 was more related to *Kat_001* than any other sequence, while MP placed AY875752 and *Kat_001* within their own monophyletic clade. Surprisingly, NJ analysis placed *Kat_001* in the taxon *S. physalifolium* with the GenBank sequence of accession number KY968822 being the closest relative. Such incongruence could be as a result of sequence sampling from GenBank, the number of sequences that are used in the construction of phylogenetic trees tends to have a positive correlation with the accuracy of the inferred tree (Heath *et al*., 2008). Indeed, it seems that, clustering of *Kat_001* with two different taxa can be explained by the number of sequences used in the analysis. The rbcl dataset, that is much larger than the ITS dataset,

does not suffer from the same effect. All the phylogenetic trees inferred from rbcL agreed that *Kat_001* is a member of *S. nigrum.* However, they had a low bootstrap value than that of ITS2.

Nevertheless, while we assume that the effect of sequence number could be the reason for higher resolution while using rbcL, we are cognizant of the fact that generally, rbcL is better at discriminating between plants of different species than does ITS sequences (Kress & Erickson, 2007). This is because the rbcl gene is maternally inherited and thus not subject to admixture, this ensures that any mutation neutral or beneficial mutation is conserved between the genus (Yao *et al*., 2010). The difference with species discrimination based on the current report occurred due to the fact that both *S. nigrum* and *S. villosum* are member of the same species complex. Many of the biological markers, including molecular markers, that are used in phylogeny are highly conserved. To farther elucidate the correct taxonomic unit to place *Kat_001,* a concatenation of both the ITS and rbcl sequences was used for analysis (Gadagkar *et al*., 2005). Previous authors have preferred to concatenate sequences from the same samples as this inevitably reduces phylogenetic noise and produces better MSA data. Here, we evaluated the approach of simply concatenating the different sequences used to infer phylogeny in the ITS and rbcL and using this for analysis. The results of the concatenated sequence agreed with the rbcL trees by placing Kat_001 within the clade containing *S. nigrum.* The agreement between rbcL and the combined set of rbcL and ITS sequences, together with morphological data strongly suggests *Kat_001* is from *S. nigrum* complex. This results were similar to those done by El-Atroush, (2020) whose observation indicated that ITS

markers are better in identifying plants at species level while Rbcl are better markers for identifying plants at generic level (El-Atroush, 2020).

The results of the nucleotide composition (table 6) support the findings of several authors. For instance the low G – C content in rbcL was observed by Arif et al., (2019) in green chireta. The small standard deviation suggests that the rbcL gene is highly conserved within closely related species, a similar conclusion was reached at by Kass & Wink, (1997). Since the rbcL is a coding sequence, the structure of nucleotide composition ought to be tied to functionality (Yao *et al*., 2019). G – C content is intimately tied to codon bias though in angiosperms its low bias is observed. This makes rbcL a suitable biomarker for identification of plant species at generic level (El-Atroush, 2020).

This is in contrast to the ITS2 G – C content that is markedly higher averaging at 61% (table 10). The current results presented in table 6 were statistically identical to those published by (Kass & Wink, 1997). Since it is known that ITS sequences are highly variable between plant families (Chupov *et al*., 2007), the small variations observed in this study testify to the fact that intraspecies identification using ITS sequences only is likely to have high resolution. This study comes to the conclusion that the plant species used in the experimentation is a subspecies of *Solanum nigrum* called *Solanum nigrum* (Subsp *villosum).* Therefore, we advise using multiple DNA barcodes when determining the species boundaries of individuals belonging to a complex, such as those found in the Solanaceae. If there is a discrepancy between the different barcodes, a concatenated data set of the same sequence may be produced.

CHAPTER FIVE

EFFECTS OF *Solanum nigrum* ON THE DIVERSITY OF CAECAL MICROBIOME IN HIGH FAT FED RATS

Abstract

Background: Diet influences gut microbiota which in turn affects both metabolism and overall human health. *Solanum nigrum (*Subsp *villosum)* is an African leafy vegetable which has previously been shown to have both nutritional and medicinal value compared to other vegetables. However, its effect on gut microbiome has not been elucidated. The objective of this study was to determine the effect of *Solanum nigrum (*Subsp *villosum)* on the diversity composition of microbiome of high fat fed rats.

Methodology: *Solanum nigrum (*Subsp *villosum)* leaves were gathered from Limuru sub-County, Kiambu County Kenya, which were later tested on Sprague Dawley high fat fed rats to determine its effect on abundance and diversity of the gut microbial community. Sprague Dawley rats were randomized and divided into 7 groups of n=5 rats each as follows: normal group (KWN1; given normal rat chow feed); positive control (KWN2; given high fat diet and a standard drug treatment-orlistat); negative control (KWN3; given high fat diet only without treatment). All of the treatment groups consumed a high-fat diet and then treated using *Solanum nigrum (*Subsp *villosum)* dose extract of 150mg/kgbw (KWN4) and 300mg/kgbw methanolic dose extract (KWN5); or 150mg/kgbw (KWN6) and 300mg/kgbw dichloromethane dose extract (KWN7). All treatments lasted 11 weeks, after which rats were sacrificed and caecum content collected for microbiome analysis.

Results: All groups had Campylobacterota, Firmicutes, Proteobacteria, Actinobacterota, Bacteriodota, Deferribacterota, Spirochaetota but with varying amounts. *Solanum nigrum*

(Subsp *villosum)* treatment groups had significantly higher composition of Campylobacterota (KWN4-99%; KWN5-55%; KWN6-92%; KWN7-90%) as compared to the negative control (no treatment KWN3-35%) ($p<0.001$) and normal control (KWN5-5%) ($p<0.001$) but similar to positive control (orlistat treatment KWN2-96%) ($p>0.05$). However, as compared to the normal control (KWN1; no fat diet and no treatment), high fat diet with supplementation of *Solanum nigrum* (Subsp *villosum)* extracts decreased the intestinal Firmicutes (KWN4-7). Other bacteria (Firmicutes, Bacteriodata, Proteobacteria and Spirochaetota) were comparable among the treatment groups (≤5%) except KWN5 which had higher Firmicutes (25%) and Bacteriodota (18%) ($p<0.001$).

Conclusion: High fat diet and *Solanum nigrum (*Subsp *villosum)* extracts caused change in the caecum microbial system of the high fat fed diet rats. Except KWN5, administration of *Solanum nigrum (*Subsp *villosum)* extract at different dosages had similar effect on the microbiome as that of the standard obesity drug (Orlistat) and could be used as an anti-obesity treatment.

5.1 Introduction

The diversified microbial community which include bacteria, virus, archaea and eukaryotic microbes are known as human microbiota since they coinhabit on human body surfaces (Hamady & Knight, 2009). However, all individuals have unique set of microorganisms on different body parts (Al Khodor *et al*., 2017b; Solt *et al*., 2011). There are various reasons for microbial diversity and these include; genetics, diet, lifestyle, geographical location, age, exposure to antibiotics or probiotics and early exposure to various microorganisms for example during gestation period, delivery , hospitalization and during feeding (Johnson & Versalovic, 2012; Ursell *et al*., 2012). Human health and disease are significantly influenced by the gut microbiome, over time microorganisms residing in the gut have evolved to very close associations with each other. Hence, they assist in food processing, protection from pathogens, vitamin synthesis, shaping the immune and nervous system, gut epithelium development and metabolism. Imbalance of gut microbiome is known as dysbiosis and may lead to host dysfunction thus contributing to the pathogenesis of a disease (Kachrimanidou & Tsintarakis, 2020b). The aim of studying microbiome is to understand eubiotic the health promoting bacteria and dysbiotic the disease causing bacteria and how the microbiota plays a role in phenotypic variants, prognosis, progression and treatment response (Al Khodor *et al*., 2017b; Odamaki *et al*., 2016).

Human gut microbiome is composed of bacteria from two phyla, bacteriodites and firmicutes (Kachrimanidou & Tsintarakis, 2020b). These complex and diverse bacteria's have led to functional expansion of the host genomes and its approximated to harbor 5- to 100 more genes when compared to the host genes (Kho & Lal, 2018). According to

various studies, microbiome changes over time due to factors such as age, for example at infancy the microbiome variation is higher compared to adults but later the variation decreases with age (Odamaki *et al*., 2016). Other factors leading to microbiome variation include use of antibiotics, change in diet, disease/ infection, puberty and pregnancy. Physical characteristics and chemical properties also influence microbiome composition of an organism (Neu, 2016; Neuman & Koren, 2017).

5.2 Methodology

5.2.1 Experimental Design

The rat groups made up of five male Sprague Dawley rats were allocated at random into the following categories: KWN I (normal control), KWN2 (positive control) was induced obesity (HF research diet) and received standard drug orlistat at a dose of 30mg/kgbw, KWN3 (negative control) was induced obesity with (HF- Research diets) and was given oral administration of water (0.1 ml/rat). Groups IV to VII (experimental groups) were induced obesity (HF- research diet) and received DCM dose extracts of *Solanum nigrum* (Subsp *villosum)* and Methanol dose extracts of *Solanum nigrum* (Subsp *villosum)* at 150 mg/kgbw and 300mg/kgbw respectively. In preparation for microbiome analysis, all caeca samples from each group were collected on the 77th day of the trial. They were then packaged according to group and stored at -80°C.

5.2.2 Total Bacterial DNA extraction

Gut samples were selected from each group and bacterial genomic DNA extracted using quick – DNA^{TM} Fecal/Soil Microbe miniprep Kit (Zymo Research, California, USA). Extraction procedure was done as follows: Seventy (70 mg) of tissue was added to a ZR bashing bead ^{TM} lysis tube of 0.5mm and thereafter 750 µl buffer was added to it. The

samples were vortexed for 40 minutes using a Votex Genic with 2ml bashing bead tubes (S5001-7). The mixture was then centrifuged at ≥ 10000 x g for 1minute and 400 µl of the supernatant transferred to a zymo-Spin ™ III-F filter in a collection tube and centrifuged at 8000 x g for 1 minute. Thereafter, Genomic lysis buffer of 1200 µl was added to the filtrate in a collection tube and centrifuged at 8000 x g. 800 µl of the filtrate was transferred into a zymo - Spin™ IICR column in a collection tube and centrifuged at 10000 x g for 1 minute. A SpinTM IICR column was then filled with 200µl of DNA pre-wash buffer, which was centrifuged at 10000 x g for 1 minute, and 500µl of g-DNA wash buffer, which was also centrifuged at 10000 x g for 1 minute. After placing Zymo SpinTM III-HRC filter in a clean collecting tube and adding 600µl of prep solution to it, the mixture was centrifuged at 8000 x g for 3 minutes. In a clean microcentrifuge tube, the eluted DNA was transferred to a Zymo SpinTM III-HRC filter before being centrifuged at 16000 x g for three minutes. The filtered DNA was then kept at - 20°C ready for library construction.

5.2.3 Library construction and Polymerase chain reaction (PCR) amplification of 16SrRNA (V3-V4 region)

The DNA sample was randomly fragmented to create the sequencing library, which was then adapter-tagged and thereafter amplified using polymerase chain reaction.

Amplification was performed on the V3 –V4 region of 16Sr RNA of the Bacterial DNA using primers F515 (5'GTGCCAGCMGCCGCGGTAA-3') and R806(5'-GGACTACHVGGGTWTCTAAT-3'). PCR Master Mix was used in amplification of 1µl of DNA with a concentration of >50ng/ µL. The detection mix for each gene contained 9.5 µl of nuclease free water, 1µl of each primer, 12.5µl one Taq rapid load 2x master mix with standard buffer and 2µl of template genomic DNA. The total volume was 25µl

reaction. Amplification was done under the following thermo cycling conditions: Initial denaturation at 95°C for 30 seconds and 35 cycles of amplification consisting of 30s at 95°C, 1 minute at 58°C for annealing, 5 minutes at 68°C for final extension. The PCR amplicons were separated by gel electrophoresis using 1 x TAE Buffer containing Gel Red Nucleic Acid at 80 volts for 1 hour on 1% agarose gel (Amplisize, Bio-Rad Laboratories), and then the results were seen via UV transillumination. One kilobase pair DNA ladder of 0.1µg/µL (Thermo Fisher Scientific California, USA) was used as a marker for the amplicon gene sizes. Quality and quantity of PCR products was determined using the Nanodrop.

5.2.4 PCR Clean- up

AMPure XP beads were put at room temperature and the amplicons centrifuged at 1000 x g at 20°C for I minute to allow condensation. The AMPure XP beads were then vortexed for 30 seconds to ensure evenly dispersal of the beads. AMPure XP beads were then added to each amplicon well and mixed using a MIDI plate at 1800rpm for 2 minutes. The amplicons were later incubated for 5 minutes at 25° C and later placed for 2 minutes on a magnetic stand together with the amplicon PCR plate, a multi channel pippete was used to discard the supernatant. The beads were later washed with 200 µl of 80 % ethanol, samples were then incubated for 30 seconds and supernatant carefully discarded in order to remove excess ethanol. The amplicon plates were then removed from the magnetic stand and thereafter 52.5 µl of 10mM Tris pH 8.5 was added, using a multi-channel pippete to each well of the amplicon PCR plate. It was gently mixed for easy resuspension of the beads and later incubated for 2 minutes at room temperature. 50 µl of

the supernatant of each plate was transferred from the amplicon PCR plate to a new 96-well PCR plate awaiting sequencing.

5.2.5 Purification, quantification and Sequencing

Beckman Coulter Genomics' Agencourt AMPure beads were used to purify the ibarcoded amplicons, and Invitrogen's Quant-iTTM PicoGreenR dsDNA Assay was used to determine their quantity. An equimolar library was built with 20 x109 molecules for individual amplicon library in order to get a comparable amount of sequences for each library, although amplicons with about a length of >600 were spiked in twice. A MiSeq system (Illumina) was used to sequence the final pooled library (20 ng/L in 100 l of Tris buffer with a pH of 8). In accordance with the TruSeq nano methodology (Illumina, FC-121-4003), the library was created by adaptor ligation and PCR using the TruSeq Nano DNA Library Prep Kit. The fragmentation step was left out. qPCR was used (Kapa Biosystems) to quantify the library. High base variability throughout the initial cycles was necessary for effective sequence clustering with Illumina sequencing. During the first cycles, we increased sequence variety by employing about 300 barcodes with different base compositions. Using the Miseq Reagent kit, the Miseq was run in 2x300 cycle configuration.

5.3 Bioinformatics analysis

The paired- end reads were analysed using Rstudio and R software version 4.1.3 pipeline, as previously described by (Bolyen *et al*., 2019). The sequences were demultiplexed using meta data file in order to obtain quality sequences. The read lengths were then trimmed to not shorter than 50 base pair and not longer than 250bp. The reads were then

denoised using DADA2 in R package and phred score of less than 30 removed (Callahan *et al*., 2016). Thereafter, subsequent analysis were done to create a phylogenetic tree, to determine relative abundance, alpha and beta diversity (Rahman *et al*., 2021).

5.4 Results

5.4.1 Taxonomy – Relative Abundance

There were diverse bacteria phyla present in the gut of the various treatment groups. The bacteria present included; Actinobacteria, Bacteroidota, Campylobacterota, Deferribacterota, Firmicutes, Proteobacteria and Sprirochaetota. The most prevalent phylum among the groups was Campylobacterota which was above 80% among the following groups KWN2, KWN4, KWN6 and KWN7, followed by Firmicutes and lastly Bacteroidota (Figure 17). The normal control group (KWN1) had the highest percentage of firmicutes (90%) compared to KWN2 (3%), KWN3 (40%), KWN4 (1%), KWN5 (25%), KWN6 (5%) and KWN7 (1%). However, the negative control (KWN3) had Bacteroidota (14%) which is non-significantly different from treatment group 5 (KWN5) (18%). One percent to 5% of proteobacteria was also present in all the experimental groups. To determine statistical significance among the phyla, Chi-square test was done whose results showed that the phylum was statistically significantly different among the treatment groups (Table 1, $p < 0.0001$). *Solanum nigrum (*Subsp *villosum)* treatment groups had significantly higher composition of Campylobacterota (KWN4-99%; KWN5-55%; KWN6-92%; KWN7-90%) as compared to the negative control (no treatment KWN3-35%) (Figure 17, $p<0.001$) and normal control (KWN5-5%) ($p<0.001$) but similar to positive control (orlistat treatment KWN2-96%) ($p>0.05$). However, as compared to the normal control (KWN1; no fat diet and no treatment), high fat diet with supplementation of *Solanum nigrum (*Subsp *villosum)* extracts decreased the intestinal Firmicutes (KWN4-7). Other bacteria (Firmicutes, Bacteriodata, Proteobacteria and

Spirochaeta) were comparable among the treatment groups (≤5%) except KWN5 which had higher Firmicutes (25%) and Bacteriodata (18%) ($p<0.001$) (Figure, 17).

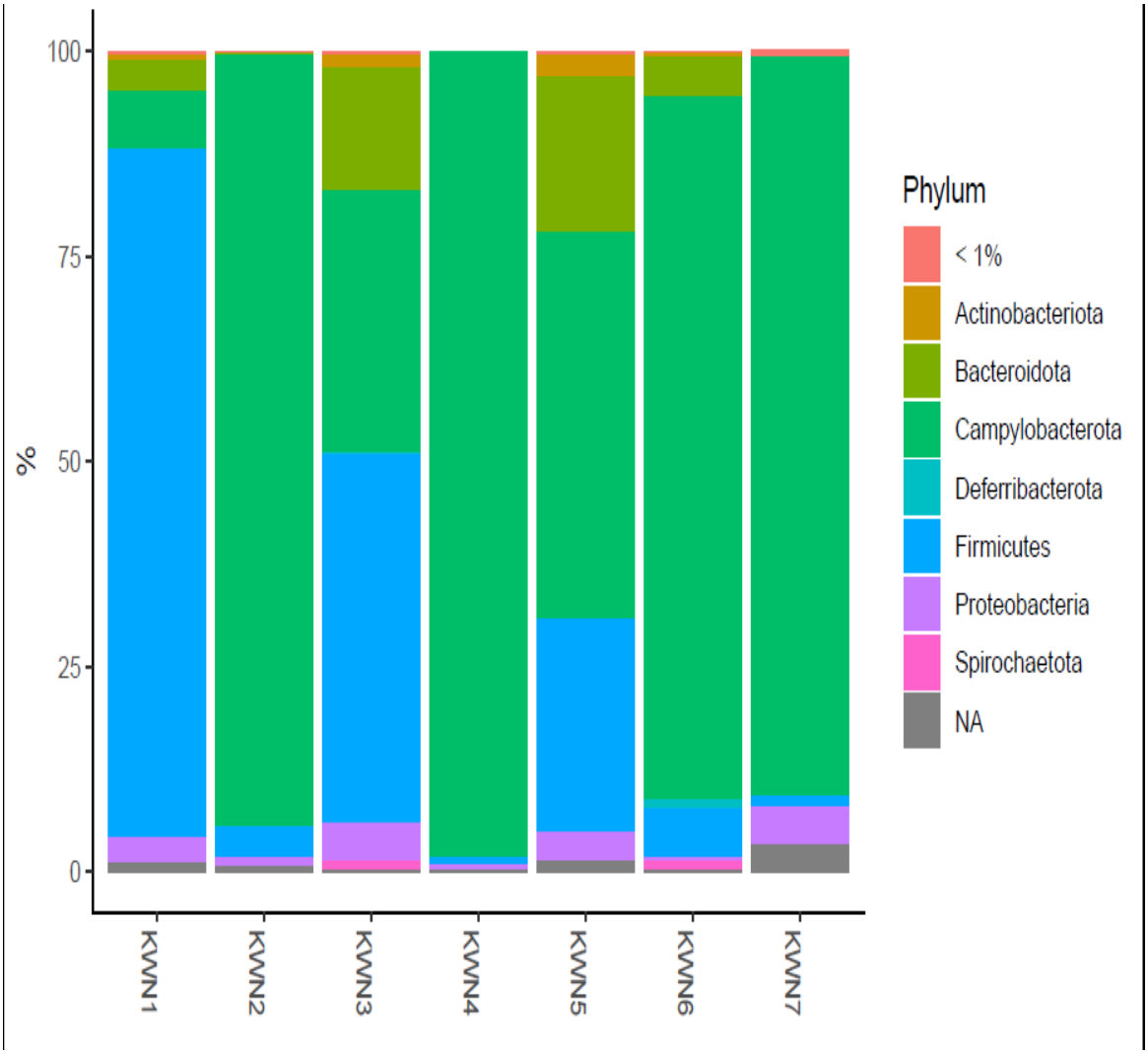


Figure 17: Relative abundance of bacterial phylum taxon among the treatment groups.

5.4.2 Alpha Diversity

Alpha diversity is a term that was first defined by Robert whittaker in 1960 as the richness in species of a particular community (Meijer *et al*., 2011). In this study alpha diversity was understood as the diversity within the treatment groups. The study showed variations within treatments groups and similarity between Shannon and Simpson diversity. The alpha diversity indicated significant variations within the treatment groups but no significant difference between KWN3 and KWN5 (See figure 18, Table 10).

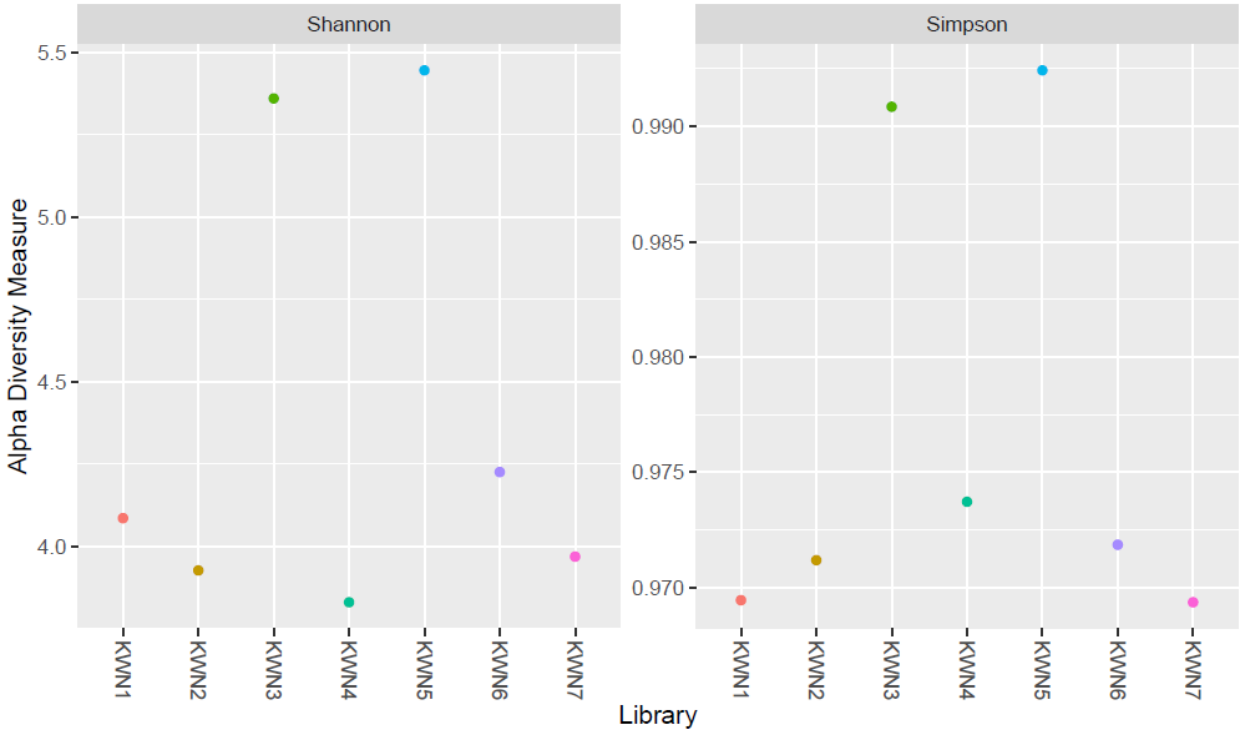


Figure 18: Alpha diversity (Shannon and Simpson) of the different groups.

Table 10: Chi-square test for the goodness of fit for the percentage of the abundance of phylum

Library	Ch-square Test	p-value
KWN1	366.88	< 0.001
KWN2	622.88	< 0.001
KWN3	156.2	< 0.001
KWN4	668.44	< 0.001
KWN5	201.4	< 0.001
KWN6	516.08	< 0.001
KWN7	550.88	< 0.001

5.4.3 Beta Diversity

Beta diversity showed significant difference between the treatment administered to various groups (Figure 19 & 20, $p < 0.001$). The results showed that treatment in group 2 (KWN2) was similar to treatment administered to group 7 (KWN7). Aditionally, a significant difference was seen between treatment administered to group KWN5 and KWN7 ($p < 0.001$).

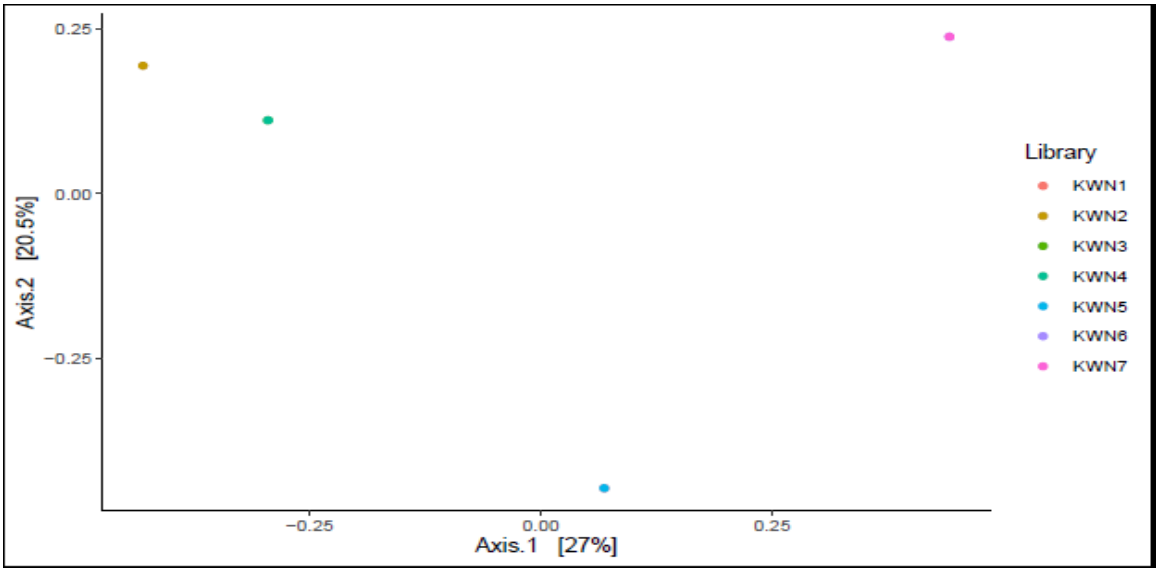


Figure 19: The figure shows beta diversity of microbial community of sprague dawley high fat fed rats under treatment

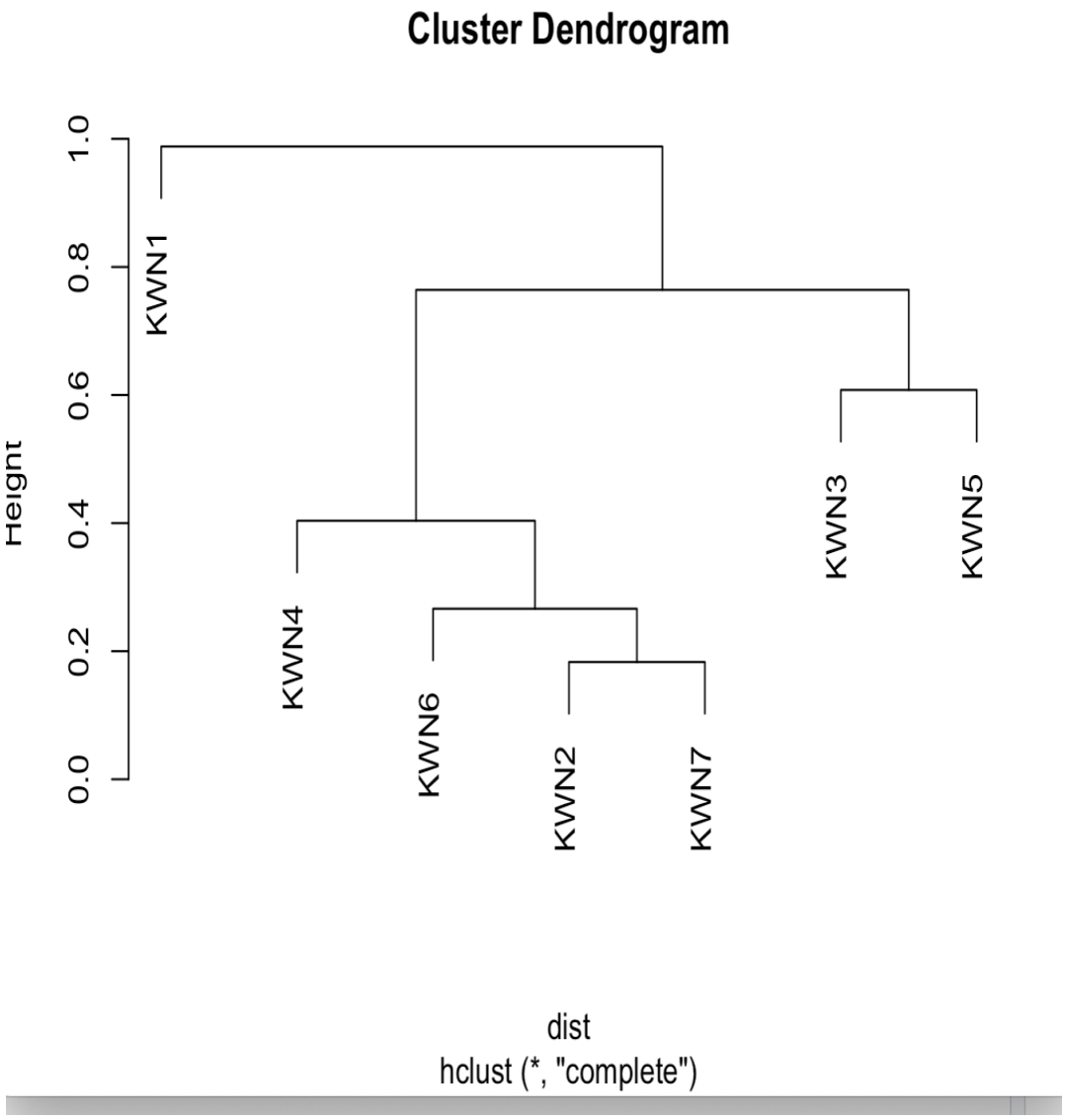


Figure 20: Dendogram describing treatment of various experimental groups

5.5 Discussion

The presence of microbiota may indicate health or disease and may vary according to diet (Kachrimanidou & Tsintarakis, 2020a; Meng *et al*., 2019; Yatsunenko *et al*., 2012). In our study, Campylobacterota, Firmicutes, Proteobacteria, Actinobacterota, Bacteroidota, Deferribacterota, and Spirochaetota were present in all groups, albeit in varied levels based on relative abundance. The presence of these bacteria has been supported by previous studies indicating that Firmicutes, Actinobacteria, Bacteroidota and Proteobacteria are found at the small intestines as well as the colon and cecal (Luo *et al*., 2021). The varying amounts observed may be due to differences in diets provided for each study group (Meng *et al*., 2019; Yatsunenko *et al*., 2012). Our study hypothesizes generally, that the presence of these firmicutes may have played a role in digestion, synthesis and absorption of nutrients, metabolism of vitamins, lipids, amino acids and short chain fatty acids as supported by previous studies (Krajmalnik-Brown *et al*., 2012; Meng *et al*., 2019; Ramírez-Pérez *et al*., 2018, Lozupone *et al*., 2012; Rinninella *et al*., 2019).

Relative proportions varied according to our study groups. For instance, our study showed increased abundance of Campylobacterota on both positive control (Orlistat treated) and *Solanum nigrum* (Subsp *villosum)* treatment groups. Orlistat treated and *Solanum nigrum* (Subsp *villosum)* may have had similar effect as traditional medicine which have been reported to increase abundance of Campylobacterota (Ma *et al*., 2014). In contrast, this study showed low abundance Campylobacterota on the negative control and was similar to previous study which indicated consumption of westernized diet

composed of high fat depletes Campylobacter (Masanta *et al*., 2013). We hypothesize that *Solanum nigrum* (Subsp *villosum)* and Orlistat may provide compounds that help proliferation of Campylobacterota.

Our study further showed high fat diet with supplementation of *Solanum nigrum* (Subsp *villosum)* extracts decreased the intestinal Firmicutes when compared to the normal control (no high fat diet and no treatment) and negative control group (high fat diet but no treatment) respectively. This is similar to previous study which indicated that introduction of plant polyphenols inhibits growth of firmicutes and bacteroidata by downregulating firmicutes to bacteroidata ratio (Parkar *et al*., 2013; Xue *et al*., 2016). Firmicutes are also known to produce butyrate which increases insulin sensitivity and is also known as an energy metabolism regulator (Resch *et al*., 2021). In contrast, although treatment KWN5 was given *Solanum nigrum* (Subsp *villosum)* methanolic extract of 300mg/kgbw, it showed a higher percentage of firmicutes and bacteroidota similar to negative control and comparable to other treatment groups. We postulate that this dose did not have a similar effect as that of orlistat and thus pancreatic lipase enzyme was not inhibited hence leading to an increase in absorption of fatty acids to the adipocytes and thus led to overweight in rats.

Other bacteria such as Proteobacteria, Actinobacterota, Deferribacterota, Spirochaetota were comparable among the treatment groups (≤5%). This study was inline with a study which indicated prevalence of proteobacteria in the intestines of normal control subjects (Shin *et al*., 2015). Proteobacteria are facultative anaerobes which make intestinal niche

favor the colonization of obligate anaerobes which are later replaced by firmicutes and bacteroidetes (Shin *et al*., 2015).

Beta diversity and the phylogenetic tree showed treatment administered was significantly different according to the clusters in our study. Generally, there were three clusters; cluster 1 (normal control KWN1), cluster 2 (KWN3 and KWN5) and cluster 3 (KWN 4-7). Cluster 1 was given a normal diet only and hence did not cluster with any other treatment group. On the other hand, cluster 2 had both the high fat diet group (negative control) and treatment group 5 which had both high fat diet and methanolic dose extract of *Solanum nigrum* (Subsp *villosum)* at 300mg/kgbw. We postulate that the extract did not have an effect on gut microbiome of the rats hence was similar to that of negative control. The other treatment groups clustered together as Cluster 3 since the effect was almost similar on the gut microbiome.

This clustering provides further support that the compounds present on the *Solanum nigrum* (Subsp *villosum)* extracts and orlistat drug had an effect on the microbial composition compared to the other clusters. We therefore, conclude that change in diet from normal diet to high fat diet significantly changed the microbiome population of caecum in rats. In addition, administration of *Solanum nigrum* (Subsp *villosum)* extract at different doses had similar effect on the microbiome as that of the standard obesity drug (Orlistat). Hence, *Solanum nigrum* (Subsp *villosum)* aids in the prevention of obesity when consuming a high-fat diet.

On the other hand, given our experimental scenario, taking into account the control groups the effect observed especially on the growth of caecal microbes was likely due to the effect of solanum extract *or* orlistat. This study recommends the use of *Solanum villosum* as a dietary supplement to prevent obesity due to its accessibility, low cost, and minimal side effects.

CHAPTER SIX

6.0 OVERALL DISCUSSION

Obesity is the abnormal increase of adipocyte cells due to proliferation and differentiation of adipocyte precusor cells to mature adipocyte cells (Jakab *et al*., 2021). This is attributed to homeostatic imbalance where by energy intake and energy expended are not proportionate (Most *et al*., 2018). In addition, it is associated with various human health problems which include cardiovascular diseases, hyperglycemia, hypertension, dyslipidemia and change in gut microbiome composition (Jayachandran *et al*., 2020). The factors involved in regulating obesity include; endocrine, genetic, nutritional, environmental, metabolic and pharmacological (Conforti & Pan, 2016).

Efforts to address obesity include various appropriate methods that have been in use for long which include: consumption of balanced diet, embracing of a healthy lifestyle through exercise and use of standard drugs such as orlistat, sibutramine, semaglutide, contrave (bupropion hydrochloride extended-release tablets and naltrexone), qsymia (phentermine), Belviq (lorcaserin hydrochloride) among others (Hursel & Westerterp-Plantenga, 2010; Tziomalos *et al*., 2009)**.** However, standard drugs have been found to have severe reactions hence researchers have embarked on natural products which have been found to be non toxic and with minimal side effects (Sun *et al*., 2016).

*Solanum nigrum (*Subsp *villosum)* species is widely used as medicine for various diseases due to its known phytochemical compounds (Hameed *et al.*, 2017). However, its effect of obesity has not been evaluated. Thus, this study used a randomized control design to

assess the anti obesity effects of *Solanum specie* on high fat fed diet *Sprague dawley* rats. This study indicated that *Solanum nigrum (*Subsp *villosum)* has anti obesity effects due to the presence of phytochemical compounds such as saponnins, phenols, flavonoids and steroids among others. We therefore postulate that the phytochemical compounds facilitated adipocytes differentiation. Adipocytes role was to maintain lipid homeostasis and energy balance by regulating free fatty acids and triglycerides storage. Additionally, increase of adipocyte cells was connected with both hypertrophy and hyperplasia and hence most natural product research foccusses on adipogenesis inhibition (Rufino *et al*., 2021). Previous studies showed that blockage of transcription factors such as CCAAT/ enhancer binding protein beta (C/ EBPβ) and Peroxisome proliferator- activated receptor gamma (PPARγ) inhibits adipocyte differentiation by decreasing lipid content in adipocyte cells (Kang *et al*., 2013; Xiao *et al*., 2010). They also lead to a decrease in serum cholesterol, low density lipoprotein- cholesterol, triglyceride, and increase in high density lipoprotein.

The current study also postulates that phytochemical compounds present in *Solanum nigrum (*Subsp *villosum)* leaves enhanced inhibition of pancreatic lipase enzyme thus inhibiting breakdown of triglycerides to fatty acids and monoglycerides leading to execretion of fats. Indeed, orlistat drug has been shown to covalently bind itself to serine pancreatic lipase active site thus blocking the absorption of the dietary fat through the intestines (Marrelli *et al*., 2013; Mulzer *et al*., 2014).

Our study also focused on gut microbial composition and according to previous studies presence of microbiota may indicate health or disease and may vary according to diet (Kachrimanidou & Tsintarakis, 2020b; Meng *et al*., 2019; Yatsunenko *et al*., 2012). In our study, all groups had Campylobacterota, Firmicutes, Proteobacteria, Actinobacterota, Bacteroidota, Deferribacterota, Spirochaetota but with varying amounts. Rats treated with *Solanum nigrum (*Subsp *villosum)* had microbiomes that were comparable to those of orlistat-treated rats. In order to determine which species of Solanum leaves exhibited the anti-obesity properties, we later went on to identify them at the molecular level.

The cryptic nature of members of *Solanaceae* makes it very difficult to assign taxonomic ranks to these members using morphological data alone (Chiarini *et al*., 2018). We therefore used two primers ITS2 and Rbcl to identify the leaves at species level. Previous studies have shown that ITS markers are better in identifying plants at species level while Rbcl are better markers for identifying plants at generic level (El-Atroush, 2020). This is because the rbcl gene is maternally inherited and thus not subject to admixture, this ensures that any mutation neutral or beneficial mutation is conserved between the species (Yao *et al*., 2010). Phylogenetic tree from ITS2 showed that the leaves were from *Solanum nigrum (*Subsp *villosum)* while rbcl indicated *Solanum nigrum* (Subsp *villosum).*

The problem with species discrimination based in the current report arose due to the fact that both *S, nigrum* and *S. villosum* are members of the same species complex. A consensus tree of the ITS2 and rbcl was also used however, concatenation analyses have been shown to produce better results (Gadagkar *et al*., 2005). The agreement between

rbcl and the combined set of rbcl and ITS sequences, together with morphological data strongly suggested the sample (*Kat_001)* is a subspecie of *S. nigrum* known as *S. villosum.*

CHAPTER SEVEN

7.0 OVERALL CONCLUSION

From this study we concluded that;

1. DCM and methanolic leaf extract reduced body mass index of high fat fed rats
2. DCM and methanolic leaf extracts reduced glucose, lipid profiles and liver function test of high fat fed diet rats
3. DCM and methanolic extracts increased phylum campylobacterota on the gut of high fat fed rats
4. *Solanum* species that showed anti obesity effects and increased campylobacterota on high fat fed rats was identified through molecular analysis as *Solanum nigrum* (Subsp *villosum).*

7.1 Overall recommendations

This study recommends consumption of *Solanum nigrum* (Subsp *villosum)* as a supplement used to reduce obesity. It acts as a pancreatic lipase inhibitor such as (orlistat drug) by blocking absorption of dietary fats through the intestines. It showed minimal side effects hence can be used in place of standard drugs such as Orlistat which are known to have severe side effects.

CHAPTER EIGHT

8.0 SUGGESTION FOR FURTHER RESEARCH

The anti-obesity effects of fractionated *Solanum nigrum* (Subsp *villosum)* compounds on high-fat-fed rats were not evaluated in our study, and the anti-obesity benefits of several related species such as; *Solanum scabrum*, *Solanum americanum*, *Solanum physallifolium*, and others, were also not identified. Additionally, we were unable to investigate *Solanum nigrum* (Subsp *villosum)* fractionated substances on the impacts of the caecal flora in rats receiving high fat diets.

To capture limitations of our study we propose further research on:

1. Anti obesity effects of fractionated compounds of *Solanum nigrum* (Subsp *villosum)* on high fat fed rats.
2. Antiobesity effects of various *Solanum* species such as *Solanum scarbrum*, *Solanum americanum*, *Solanum physallifolium* among others.
3. Determining effects of fractionated compounds on caecal microbiome of high fat fed rats.
4. Isolation and characterization of important obesity related microorganisms

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APPENDICES

Serial No: KyU-AIC 2022/100

Kirinyaga University

Certificate

This is to certify that *Kathryn Nderitu* from ***University of Nairobi,*** **Presented** a paper titled: **Solanum nigrum *L. Show Anti-Obesity Effects on High-Fat Diet-Fed Sprague Dawley* Rats *in a Randomized Study*** during the 5th Annual Virtual International Conference under the theme"***Re-Engineering Leadership for Sustainable Development***", *held on March 24-25, 2022 at Kirinyaga University, Kenya.*

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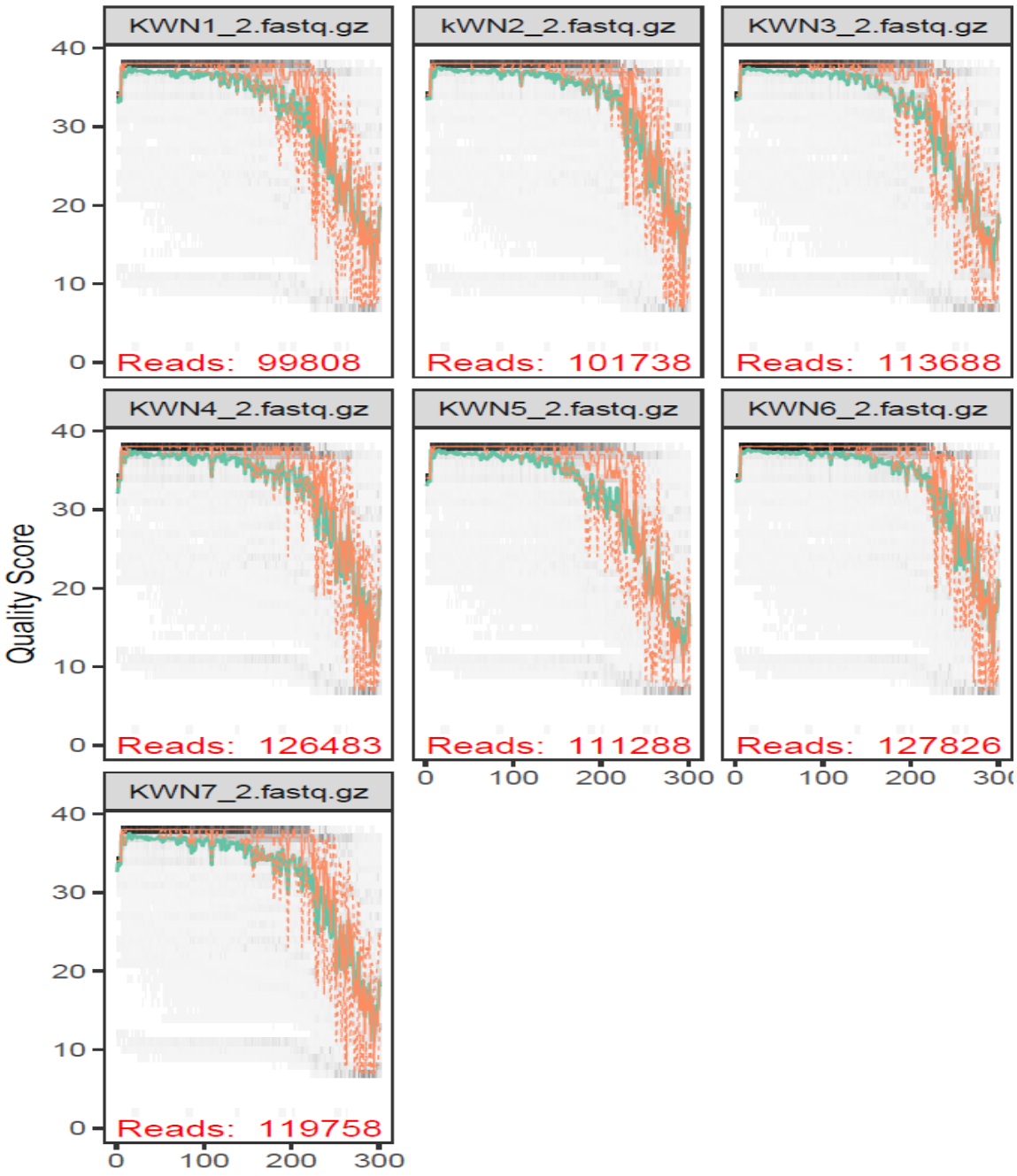
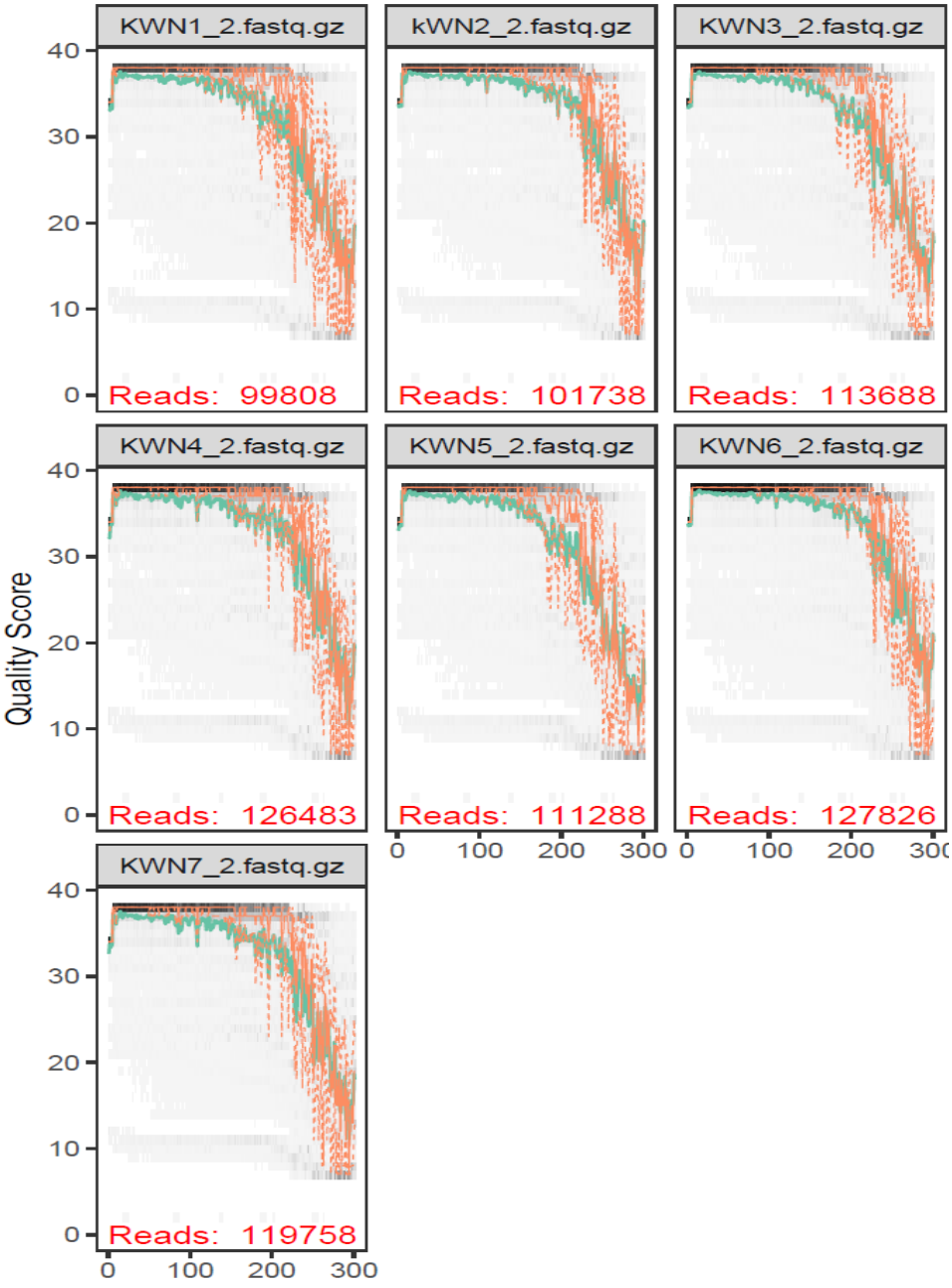
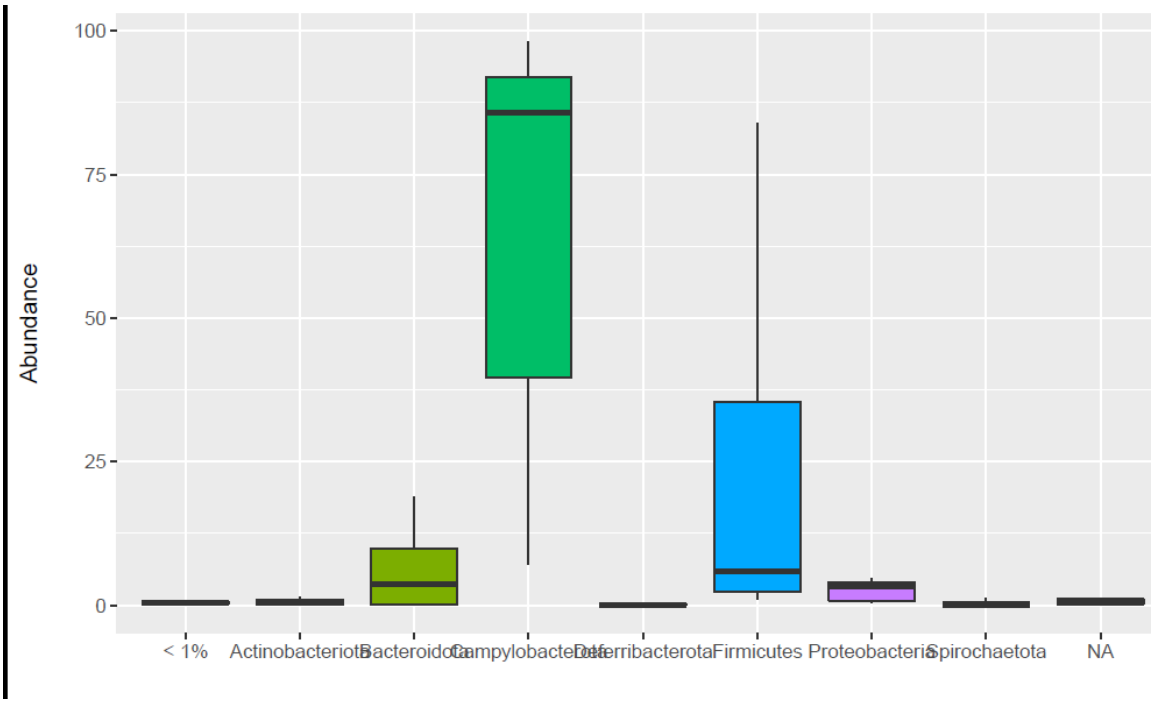
Appendix 1: Presented my research work in a conference



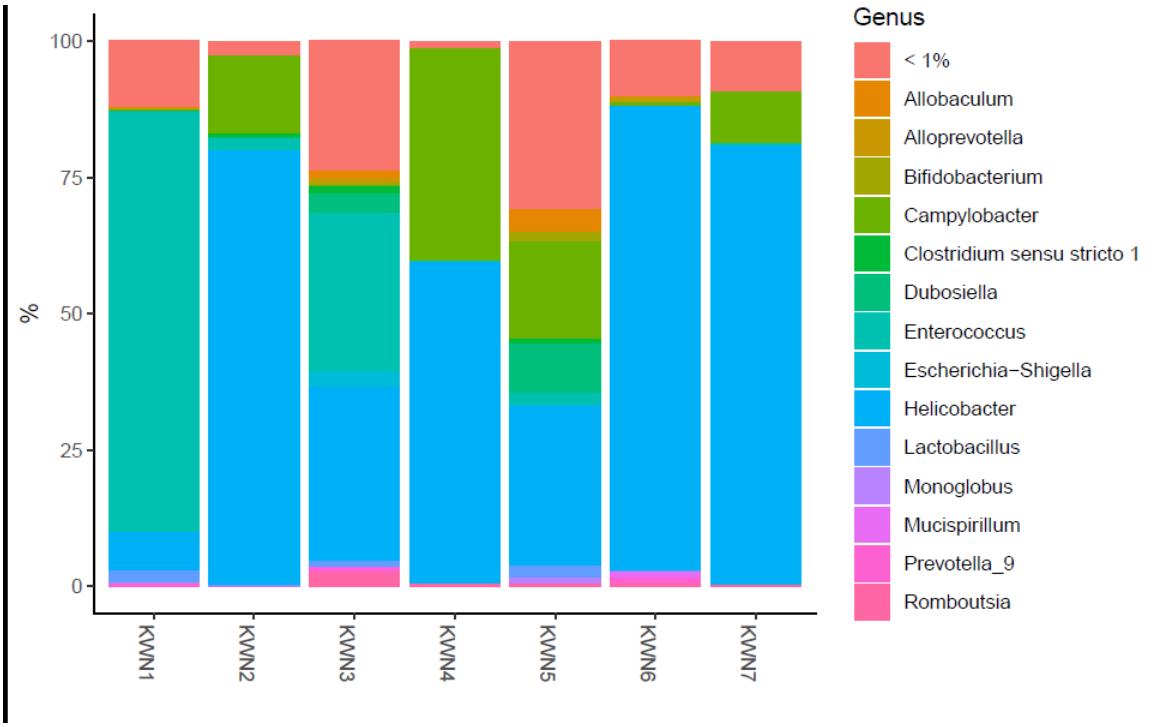
Appendix 2: Gut microbiome forward reads



Appendix 3: Gut microbiome reverse reads

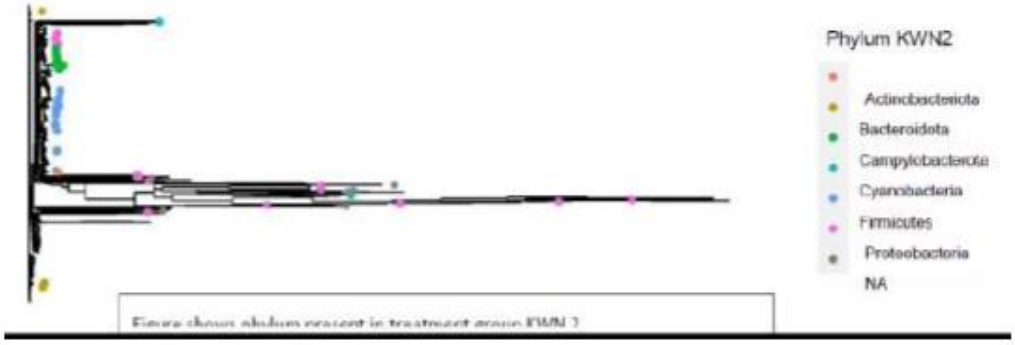


Appendix 4: A box plot showing abundance of bacterial phylua taxon among the treatment groups



Appendix 5: Relative abundance of bacterial genus taxon among the treatment groups.

Figure shows phylum present on the normal control KWN1



Appendix 6: Bacterial phylum present on the gut of KWN1 and KWN2

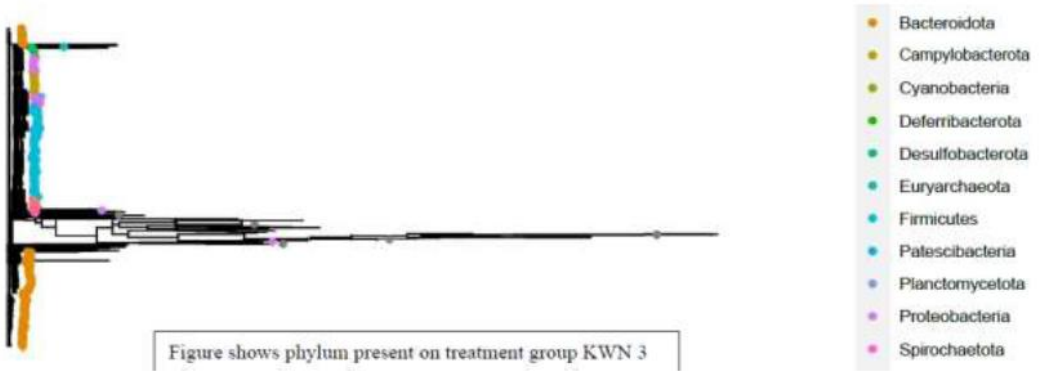


Figure shows phylum present on treatment group KWN 3

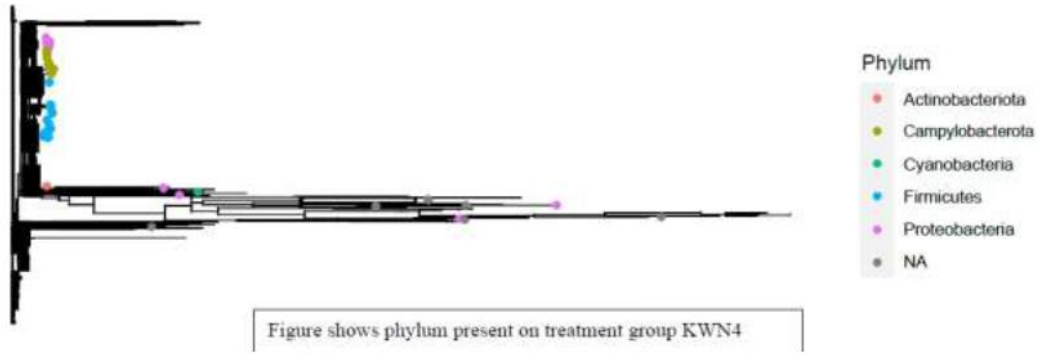


Figure shows phylum present on treatment group KWN4

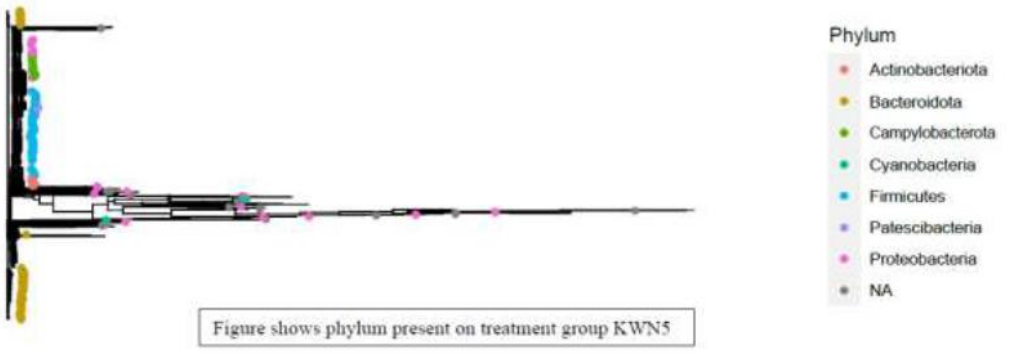


Figure shows phylum present on treatment group KWN5

Appendix 7: Bacterial phylum present on the gut of KWN3, KWN4 and KWN5

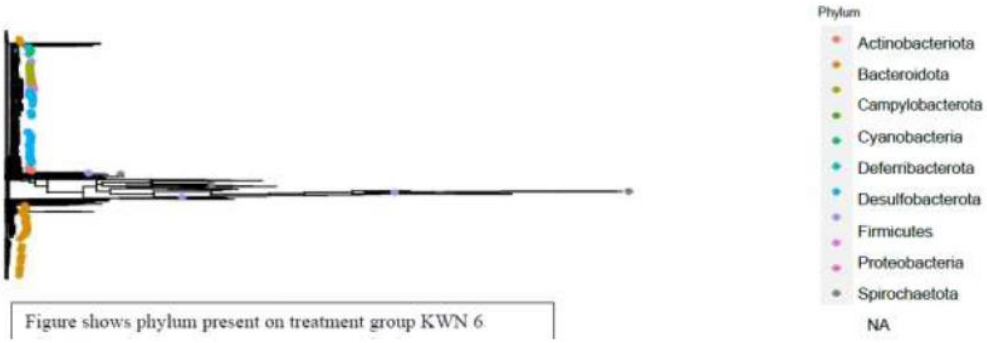


Figure shows phylum present on treatment group KWN 6

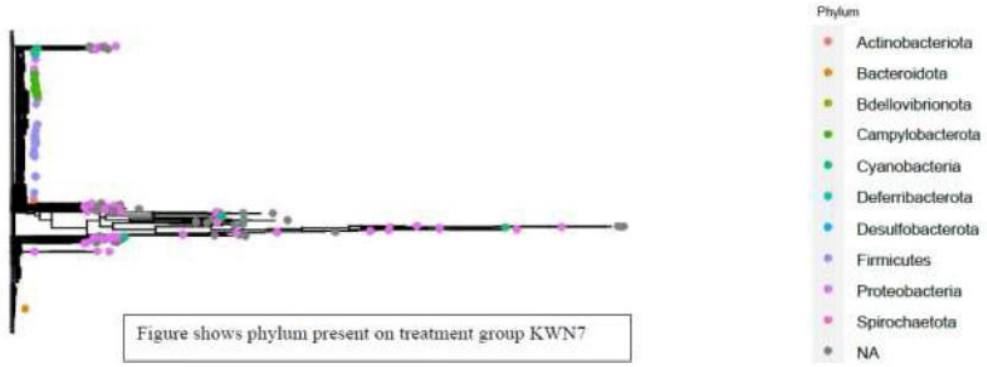
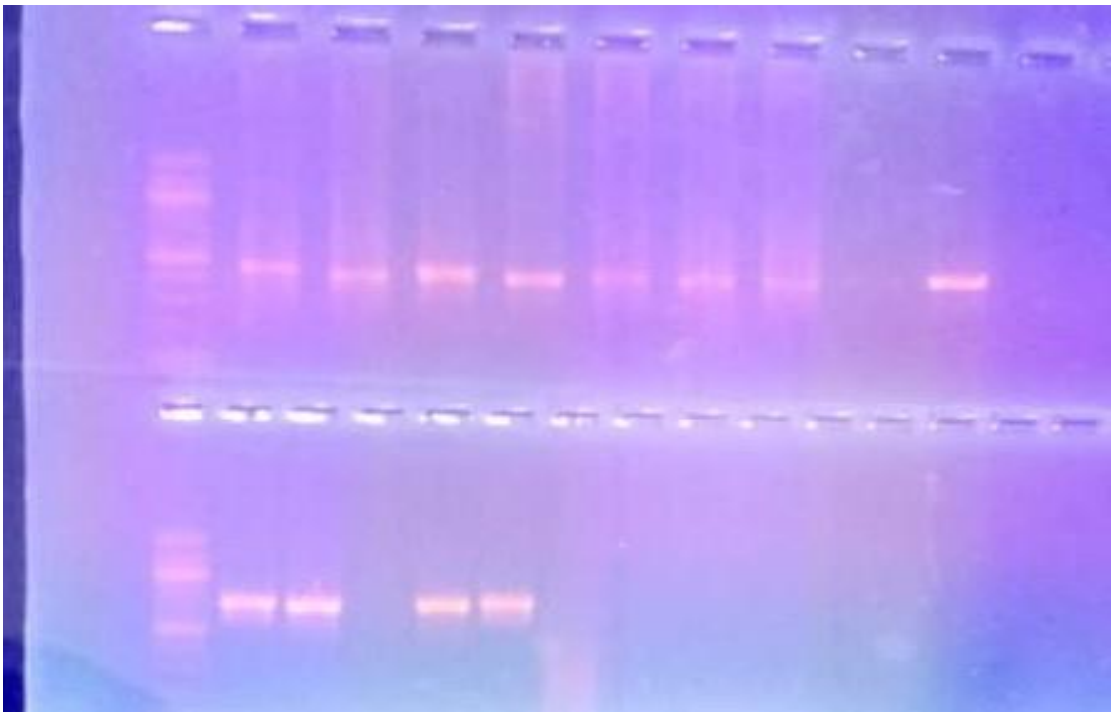


Figure shows phylum present on treatment group KWN7

Appendix 8: Bacterial phylum present on the gut of KWN6 and KWN7



Appendix 9: Duplicate samples of Plant DNA using ITS2 and rBCL Markers (bottom section) and seven samples of bacterial DNA and a positive control (upper section).