STUDY OF PHYTOCHEMISTRY, TOXICITY AND ANTIHYPERTENSIVE ACTIVITY OF *CARPOBROTUS EDULIS* AQUEOUS LEAF EXTRACT

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DECLARATION

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

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DEDICATION

The work is dedicated to the Sikazenge Family, for their unwavering moral support throughout the study period.

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ABBREVIATIONS

˚C	Degrees Celsius
µL	Microlitres
µmol/L	Micromoles per Litre
2D NMR	Two Dimensional Nuclear Magnetic Resonance Spectrometry
ABTS	2, 2'-Azino-Bis-3- Ethylbenzothiazoline 6-Sulfonic Acid
ACE	Angiotensin Converting Enzyme
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
DPPH	2, 2-Diphenyl-1- picrylhydrazyl
fL	Fetolitre
g/dL	Grams per Decilitre
g/L	Grams per Litre
GC-MS	Gas Chromatography-Mass Spectrometry
GPS	Global Positioning System
IU/L	International Units per Litre
LC/ESI-MS/MS	Liquid Chromatography Electrospray Ionisation Tandem Mass Spectrometry
LD_{50}	Medium Lethal Dose
MCH	Mean cell hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume

MDGD	Monogalactosydiacylglycerol
MDR	Multi-Drug Resistant
MS	Mass Spectrometry
NCDs	Non Communicable Diseases
NHANES	National Health and Nutrition Examination Survey
OECD	Organization of Economic Cooperation and Development
PCV	Packed cell volume
RAAS	Renin Angiotensin Aldosterone System
RBC	Red blood cells
SEM	Standard Error of the Mean
SNS	Sympathetic Nervous System
SPSS	Statistical package for the Social sciences
Thromb.	Thrombocytes
U.S.A	United States of America
WBC	White blood cells
W.H.O	World Health Organisation

ABSTRACT

Carpobrotus edulis is a medicinal plant widely used as a folk medicine in Southern Africa, to treat hypertension and other ailments. Despite its extensive use in herbal medicine, there is no documented scientific evidence corroborating its safety or any that validates its therapeutic use in hypertension treatment. Therefore, the aims of this study were to investigate the antihypertensive effects of aqueous extracts of *Carpobrotus edulis* in fructose-induced hypertensive rat models and also to determine the toxicological effects of both acute and subacute exposure to aqueous extracts of *Carpobrotus edulis* in Sprague Dawley rats. Aqueous extraction and phytochemistry of *Carpobrotus edulis* leaves was performed by using standard routine methods. The acute and subacute toxicity evaluation of the aqueous extract of *Carpobrotus edulis* was done using established methods (OECD guidelines). In acute toxicity testing, a single oral exposure of the extract was given to three healthy female Sprague Dawley rats at the four fixed dose levels of 300, 600, 1200 and 2000 mg/Kg body weight. The rats were observed clinically for any signs of toxicity for a period of 24 hours. These Sprague Dawley rats were then kept for 14 days and were weighed weekly. On the fourteenth day, the animals were euthanized using chloroform and gross necropsy was performed on all animals. For the subacute toxicity study, thirty-two (32) Sprague Dawley rats of both sexes equally represented, were grouped into three treatment groups (A-C) and one negative control group D of eight animals each. Group A received 100 mg/Kg of the extract; Group B received 300 mg/Kg while Group C received 1000 mg/Kg of the extract for 28 days. The rats were weighed prior to dosing at weekly intervals and the weight of each rat recorded separately. Feed and water consumption were also measured on a weekly basis. After the 28th day, the effect of *Carpobrotus edulis* on organs, haematological and biochemical parameters were assessed. The haematological parameters which were determined included Total

Red Blood Cell count, Red Blood Cell Distribution Width, Total Leucocyte Count, Haematocrit, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration, Platelet counts and Mean Platelet Volume. The biochemical parameters which were determined included Alanine Aminotransferase, Aspartate aminotransferase, Alkaline phosphatase, Albumin, Total protein, Blood Urea Nitrogen and Creatinine. In the evaluation of antihypertensive efficacy, thirty Sprague Dawley rats were divided into five groups (A-E) of six animals each. Hypertension was induced by using fructose (w/v) 20% drinking water for 21 days in Groups A-D. A non-invasive tail-cuff system (BIOPAC® System Inc., CA) was used to measure systolic and diastolic blood pressure twice weekly. Group A and Group B then received 300 mg/Kg and 1000 mg/Kg of aqueous extract of *Carpobrotus edulis* respectively for 14 days. Captopril (50 mg/Kg) was used as a standard antihypertensive drug in a positive control Group C. Group D received only 20% (w/v) Fructose solution for the entire experimental period while Group E was the negative control and it did not receive any treatment. The obtained data was fed into the Statistical Package for Social Sciences software for further statistical analysis. Statistical analysis was carried out using one-way ANOVA in order to compare group means of measured parameters and a 95% level of significance ($p \leq 0.05$) was used in the analysis. Phytochemical screening revealed the presence of flavonoids, anthraquinones, alkaloids, terpenoids, saponins, tannins and glycosides. The LD_{50} of the aqueous extract of *Carpobrotus edulis* was estimated to be above 2000 mg/Kg. On subacute toxicity testing, there were no significance differences ($P<0.05$) on all of the investigated parameters in all experimental groups. The aqueous extracts of *Carpobrotus edulis* reduced blood pressure significantly in Fructose induced hypertensive rats. It is therefore concluded that the aqueous extracts of *Carpobrotus edulis* have potential antihypertensive effects and are safe. There is however a need for a rigorous toxicological

evaluation to guarantee safety of the medicinal plant. Lastly, it is recommended that further investigations should be done on identity of the active molecules and mechanism of action of the herbal extract in order to develop new antihypertensive medicines.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Hypertension, also referred to as high blood pressure, is a crucial emerging public health issue in Sub-Saharan Africa and other developing countries. Hypertension-associated mortalities have recently become more common in developing countries. A study on global burden of hypertension indicates that over 25% of the total adult population in the world was hypertensive in the year 2000 and the ratio will increase to 29% by 2025 (Praveen and Halesh, 2010).

Hypertension is classified according to cause as primary or secondary hypertension. Primary hypertension arises due to unexplainable causes while secondary hypertension arises due to underlying medical conditions such as chronic kidney disease (Kanbay *et al.,* 2008). Hypertension is best regulated through the diet and regular exercises. The lifestyle modification is commonly known as the standard prophylaxis and/or first line treatment for hypertension (Lapi *et al.,* 2013).

Different classes of conventional antihypertensive drugs are available for the treatment of hypertension. Adherence to antihypertensive therapy, a chronic condition, is essential for proper management of the condition in patients. The cost of drug therapy especially in resource-poor settings is a major setback of adherence to long term medications for hypertension (Mueke, 2013; Weber *et al.,* 2014). Hypertensive patients therefore seek alternative means to alleviate suffering through use of herbal medicines from traditional healers. A variety of traditional medicinal plants are reported to have been used in hypertension management all over the world (Grant *et al.,* 2012).

Carpobrotus edulis, commonly known as *Igcukuma* by Xhosa community of Zimbabwe, is an edible ground cover plant that is widely used to treat hypertension in Southern Africa (Omoruyi *et al.*, 2012). It is also used by the traditional therapist in the Eastern Cape Province in South Africa in the treatment of diabetes mellitus, tuberculosis, sores, constipation and intestinal worms (Buwa and Afoloya, 2009). However, there is a knowledge gap with regard to scientific validation on medicinal use and safety of *Carpobrotus edulis*. It is therefore of greater importance to validate the therapeutic uses of *Carpobrotus edulis* as well as evaluating its safety profile.

1.2 Research problem and Justification.

The rationale for the use of *Carpobrotus edulis* in hypertension treatment is based largely on long-term experience on traditional medicine practitioners' experience (Omoruyi *et al.*, 2012). Published work on antihypertensive efficacy and toxicity of *Carpobrotus. edulis* is scanty despite the plant being widely used in traditional medicine. The extensive use of *Carpobrotus edulis* in traditional medicine may be posing a toxicological hazard to the exposed population. This study will investigate the phytochemistry, toxic properties and antihypertensive efficacy of *Carpobrotus edulis* growing in Zimbabwe.

1.3 Objectives:

1.3.1 General objective of the study

The general objective of the study was to perform phytochemical screening, investigate the antihypertensive effects of *Carpobrotus edulis extracts* and to determine its toxicological effects.

1.3.2 Specific objectives

The specific objectives of this study are;

i. To screen for presence of major phytochemical constituents of *Carpobrotus edulis* extracts.
ii. To determine the acute toxic effects of single oral exposure to aqueous extracts of *Carpobrotus edulis* by estimating their LD_{50} in Sprague Dawley rats.
iii. To determine the subacute toxic effects of repeated oral exposure to aqueous extracts of *Carpobrotus edulis* in Sprague Dawley rats.
iv. To determine the antihypertensive efficacy of aqueous extracts of *Carpobrotus edulis* in Fructose-induced hypertensive Sprague Dawley rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter covers literature on the ethno-medical uses and Pharmacological effects of *Carpobrotus edulis.* The chapter also gives details on hypertension, aetiology and pathophysiology of hypertension as well as the epidemiology and management of hypertension. Relevant literature review on phytochemical screening and toxicity studies are also covered in this chapter.

2.2 Background and taxonomy of *Carpobrotus edulis*

Traditional medicine is the most affordable and easily accessible treatment method in the primary healthcare system in developing countries. More so, traditional medicines are culturally acceptable in various societies (Peltzer *et al.*, 2008). Studies have revealed that about half of the African population use traditional medicine regularly (Mahomoodally, 2013; Oreagba *et al.*, 2011). Traditional medicine in developing Countries therefore contributes directly to the socioeconomic status of the rural and urban communities. Africa has an extraordinary richness in its flora, amounting to several thousands of species. Researchers suggest that about 10% of Africa's flora is of medical importance and some of the plant species have been studied in biomedical research (Gurib-Fakim & Mahomoodally, 2013). The genus *Carpobrotus* (Aizoaceae) has about 12 to 20 species which are very similar in appearance and their correct identification should be done by a taxonomist (Roiloa *et al.*, 2010). Most of these are endemic to South Africa but there are at least four Australian species and one South American species (Maltez-Mouro *et al.*, 2010; Novoa *et al.*, 2012). Some species from this genus have important validated medicinal properties which can provide leads to drug development. Most of the species

which are native in South Africa are used in traditional medicine and some of their Pharmacological activities have been studied (Van Wyk, 2008). *Carpobrotus mellei* has antimicrobial activities against *Pseudomonas aeruginosa* and *Staphylococcus aureus.* A study by Springfield *et al.,* (2003) showed the antimicrobial activity of *Carpobrotus muirii* and *Carpobrotus quadrifidus* extracts against *Staphylococcus aureus* and *Mycobacterium smegmatis*. The traditional uses, phytochemical composition and pharmacological activities of *Carpobrotus edulis* are reviewed.

The succulent plant *Carpobrotus edulis* commonly known as sea fig (Figure 2.1) is a perennial ground creeping species native from South Africa that invades coastal habitats in many parts of the world. The plant was originally called *Mesembryanthemum edule* and it was renamed by Brown in 1926 and by Bolus in 1927 to *Carpobrotus edulis* (O'Rourke & Lysaght, 2014). *Carpobrotus* mainly inhabits sandy coastal habitats and can also be found inland in sandy to marshy places (Campoy *et al.*, 2018). There is a continued significant risk of deliberate introduction as the plant is propagated for their ornamental properties.

Taxonomic Tree

Domain: Eukaryota

Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Caryophyllales
Family: Aizoaceae
Genus: Carpobrotus
Species: *Carpobrotus edulis*

Figure 2.1: A picture of flowering *Carpobrotus edulis* plant taken from Cannock Gardens in Mt. Pleasant District, Harare (Picture taken by Mudimba Toonse).

2.3 Traditional uses of *Carpobrotus edulis*

Carpobrotus edulis has been used widely in Southern Africa as a traditional medicine for a wide range of ailments. The fruits, leaves and flowers are medicinally used in different forms. Mostly, the plant's leaves, fruits or flowers are chewed raw or boiled in water and orally taken as a medicine for various bacterial and fungal infections (Steenkamp *et al*., 2007). In Sub Saharan Africa, the boiled leaves of *Carpobrotus edulis* are used in the treatment of tuberculosis and other respiratory infections (Buwa & Afolayan, 2009). *Carpobrotus edulis* leaves may have analgesic effect; the leaves are boiled in water for toothache and earache treatments. However, their antimicrobial effect may be responsible for the analgesic effect because most toothaches or

earaches are caused by various colonizing microbes. The leaf juice, however, has traditionally proved to be effective in soothing pain caused by spider and tick bites (Ibtissem *et al*., 2012). Facial eczema, wounds, burns and various skin conditions are treated by either chewing *Carpobrotus edulis* leaves or by drinking boiled leaves (Van Wyk, 2011). Topical use of *Carpobrotus edulis* extracts in traditional medicine is not very common. The Xhosa-speaking people in the coastal areas of the Eastern Cape Province of South Africa commonly administer aqueous and alcohol extracts to patients for the management of HIV/AIDS associated diseases (Omoruyi *et al*., 2012; Wilfred *et al.,* 2011). The leaves also have an acerbic antiseptic fluid orally taken as mouth gags for sore throat and mouth infection treatments (Van Wyk *et al*., 2008). The leaf is also boiled for treatment of intestinal worms, dysentery, diarrhoea and different stomach aches (Semenya & Maroyi, 2012; Bisi-Johnson *et al*., 2010). In Tunisia, the leaves are boiled in water for treatment of sinusitis, chilblains and vaginal thrush (Ibtissem *et al.,* 2012). *Carpobrotus edulis* also seems to be important in the treatment of chronic non-communicable diseases like hypertension and diabetes mellitus (Rocha *et al*., 2017; Davids *et al*., 2016; Al-Faris *et al.,* 2010). A detailed literature review on hypertension was done in order get further information.

2.4 Hypertension

Hypertension or high blood pressure is a chronic medical condition in which there is persistent elevation of blood pressure (> 120/80 mmHg) in the blood vessels. Blood pressure arises from stronger heart contraction and/or from increased peripheral resistance. Systolic parameters measure the force of contraction of the heart ventricle while the diastole measures their relaxation. Clinical state of hypertension in human beings has been categorized as shown in Table 2.1.

Table 2.1: Categories of hypertensive states in humans

Stage of hypertension	Blood pressure parameters
Normal	Less than 120/80 mm Hg
Elevated	Systolic between 120-129 and diastolic less than 80;
Stage 1	Systolic between 130-139 or diastolic between 80-89
Stage 2	Systolic at least 140 or diastolic at least 90 mm Hg;
Hypertensive crisis	Systolic over 180 and/or diastolic over 120,

Source: American College of Cardiology Foundation, 2018.

2.4.1 Etiology and pathophysiology of hypertension

Hypertension can be classified as either primary or secondary depending on the cause. About 90–95% of hypertension is primary hypertension (e Silva and Flynn, 2012). Primary hypertension arises from unexplainable cause or its cause as being due to increased sodium intake and decreased potassium intake. Secondary hypertension results from a particular underlying condition with a well-known mechanism, such as chronic kidney disease and adrenal diseases (Kanbay *et al.*, 2008). There are multiple mechanisms that maintain normal blood pressure. These mechanisms are the renin-angiotensin-aldosterone system (RAAS), endothelial function and sympathetic nervous system (SNS). Derangement of any of the systems then leads to hypertension.

2.4.1.1 Renin-angiotensin-aldosterone system

The RAAS system, activated by the SNS stimulation and glomerular hypoperfusion, plays a very critical role in hypertension development. These two stimuli trigger a cascade of events which lead to the release of a potent vasoconstrictor, Angiotensin II. Angiotensin Converting Enzyme (ACE) is a pharmacologically important protein in the cascade. It cleaves the impotent

Angiotensin I to a potent Angiotensin II. Aldosterone is secreted in response to low salt intake. Aldosterone increases sodium reabsorption and water retention resulting in further increase of blood pressure (Sarzani *et al.,* 2008).

2.4.1.2 Sympathetic nervous system

Sympathetic stimulation of the kidneys, heart and peripheral vasculature result in an increased fluid retention, increased cardiac output and increased vascular resistance (Sobotka *et al.,* 2012). It increases renin secretion thereby activating the RAAS system which finally causes a high blood pressure.

2.4.1.3 Endothelial function

The major underlying mechanism for endothelial dysfunction seen in high blood pressure is the decrease in the availability of nitric oxide (NO), a consequence of increased oxidative stress in these patients. Inhibition of endothelium-derived nitric oxide synthase leading to unavailability of nitric oxide results in hypertension in humans (e Silva and Flynn, 2012.).

2.4.2 Epidemiology of Hypertension

2.4.2.1 The global burden

Hypertension is a worldwide problem affecting about 15-20% of all adults (Yang *et al*., 2008). A global burden of hypertension study has indicated that nearly a quarter of the adult population had hypertension in 2000 and the proportion will rise to 9% by 2025. A study done on the National Health and Nutrition Examination survey NHANES (2005-2006) revealed that a third of the adult population in the USA had hypertension, which is about 29% of the adult population (Ostchega *et al.,* 2008). The global burden of hypertension continues to grow yearly. Hypertension is the third largest killer in the world responsible for about 13% of all deaths in the

world (Yang *et al.*, 2008). The seriousness of hypertension is evident by the high prevalence and the associated increase in heart disease complications.

2.4.2.2 Hypertension in developing countries

Hypertension has emerged as a serious Public Health problem in Sub Saharan Africa and other developing countries. The prevalence of hypertension is estimated at 22.9% in developing Countries and 37.3% in developed countries (Mutowo *et al.*, 2015). In Zimbabwe, hypertension was responsible for an estimated 21% of the total deaths in the year 2008 (Mutowo *et al.*, 2015). According to statistics published by the WHO (2013), hypertension deaths reached 0.90% of Kenya annual deaths. These statistics analysed the twenty main causes of death in Kenya and hypertension was ranked position eleventh behind HIV/Aids, strokes, heart diseases, Tuberculosis road accidents and violence (Mueke, 2013). The above WHO (2013) Kenya Statistics show the prevalence of hypertension in rural Kenya is 21% compared to 19% in rural areas of Nigeria.

2.4.3 Management of hypertension

2.4.3.1 Non-pharmacologic management of Hypertension

Lifestyle modification is important in management of hypertension. This involves healthy diet, weight reduction and regular aerobic exercise. Smoking cessation and low alcohol consumption have been documented to be very important in non-pharmacological management of hypertension (Briasoulis *et al.*, 2012). A long term low sodium diet is effectual in decreasing blood pressure and its potential effectiveness can be equated to a single medication regime (Lapi *et al.*, 2013).

2.4.3.2 Conventional drugs for management of hypertension

There are several classes of antihypertensive medications available for hypertension management.

Angiotensin Converting Enzyme (ACE) inhibitors prevent the formation of angiotensin II from Angiotensin I and also reduce the breakdown of the vasodilator bradykinin thereby increasing its availability. Captopril, Ramipril and benazepril are some of the examples of ACE inhibitors. These ACE inhibitors are usually used in monotherapy or in combination with diuretics and calcium channel blockers (Lapi *et al.,* 2013; Gradman *et al.,* 2010). The common adverse effect of ACE inhibitors is severe hypotension which occurs after initial doses in hypovolemic patients. ACE inhibitors are also known to cause hyperkalemia, acute renal failure and angioedema (Izzo & Weir, 2011). Captopril causes neutropenia and proteinuria when given in high doses to patients with renal insufficiency.

Calcium channel blockers bind to the L-channels of endothelial smooth muscle cells and disrupt the influx of calcium into the muscle cell thereby preventing the contraction of cardiac myocytes and smooth muscle cells (Costanzo *et al.*, 2009). They are categorised into dihydropyridines and non-dihydropyridines. Dihydropyridines reduce blood pressures primarily by direct vasodilation and reduction of systemic vascular resistance but non-dihydropyridines decrease both the heart rate and myocardial contraction. Nifedipine and amlodipine are examples of dihydropyridines calcium channel blockers while verapamil and diltiazem belong to the non-dihydropyridines category. These calcium channel blockers are known to significantly reduce the risk of stroke in patients with hypertension (Costanzo *et al.,* 2009). Calcium channel blockers, especially the dihydropyridines cause adverse effects related to excessive vasodilation (Chandra & Ramesh, 2013). The symptoms shown are dizziness, headache, hypotension and nausea.

Diuretics increase renal sodium and water excretion thereby reducing the volume of water in circulation. Scientific evidence suggests that diuretics reduce the risk of stroke and improve cardiovascular outcomes. Spironolactone, a potassium sparing diuretic, decreases the risk of mortality in hypertensive patient with heart failure (Shafi *et al.,* 2008). Spironolactone induces adverse effects such as gynecomastia, impotence and menorrhagia. Thiazide diuretics are preferred in clinical practice, loop diuretics are used in association with potassium sparing diuretics to reduce the risk of hypomagnesemia and hypokalaemia and hypomagnesaemia (McAlister *et al.,* 2009). These conditions are known to thwart diuretic therapy. Erectile dysfunction is the common adverse effect of the thiazide-class diuretics (Joshi *et al.,* 2010). This class of diuretics also induce hyperuricemia which can cause gout.

Beta-blockers are not preferred as initial treatment in hypertension but there are of first priority in patients with history of myocardial infarction and heart failure (Rosenson *et al.,* 2018). Beta-Blockers lower the blood pressure by reducing cardiac output, suppressing renin production and inhibiting central sympathetic activities. Atenolol, metoprolol and nevibolol are examples of cardioselective beta-blockers while propranolol and labetalol are examples of nonselective beta blockers. Beta-blockers cause adverse effects on lipid and glucose metabolism when used alone or in combination with diuretics (Rizos & Elisaf, 2014). They also induce coronary spams through activation of alpha-1 receptors. Sudden discontinuation of beta-blockers induces withdrawal symptoms such as angina pectoris and hypertensive attacks, therefore the dose should be gradually reduced before withdrawal. Concomitant use of beta-blockers with calcium channel blockers is known to induce bradycardia and heart failure (Pea, 2017).

Alpha-blockers selectively block alpha 1-receptors on the smooth muscles causing vasodilation. They are usually used as second line treatment for high blood pressure. Alpha 1-blockers are

used to control blood pressure before surgery on pheochromocytoma (Ramachandran & Rewari, 2017). Prazosin, Terazosin and Doxazosin are examples of alpha blockers used in treatment of hypertension. The common adverse effects of these drugs include dizziness, palpitations and syncope due to orthostatic hypotension (Domínguez-Domínguez & Calderón-Ospina, 2015). Therefore, the administration of alpha-1 blockers should be started at a low dose with gradual increase.

Centrally acting sympatholytic drugs inhibit sympathetic activities by stimulating alpha 2-receptors in the vasomotor centre of the medulla oblongata which leads to reduction of blood pressure. Centrally acting antihypertensive drugs are usually used when other antihypertensive drugs are not tolerated (Ming *et al.,* 2011). Guanabenz, clonidine and Methyldopa are examples of centrally acting antihypertensive drugs. These drugs have lost their clinical importance because they have unfavourable side effects mediated by alpha 2- adrenoceptors. The adverse effects caused by the drugs include sleepiness, liver dysfunction, dizziness, thirst, malaise and impotence. Methyldopa is usually used for the treatment of pregnancy-induced hypertension (Brown & Garovic, 2014).

Classic vasodilators dilate blood vessels by directly acting on the vascular smooth muscle. Hydralazine is used to treat pregnancy-induced hypertension. It is also used in hypertensive emergencies because of its short onset of action (Vidt, 2001). Classic vasodilators have various adverse effects which include headache, palpitation, tachycardia and fulminant hepatitis. When classic vasodilators are used continuously, systemic lupus erythematosus-like symptoms may appear (Dalle Vedove *et al.,* 2012).

Angiotensin II Receptor blockers reduce blood pressure by blocking the activation of angiotensin II receptors found in smooth muscle cells of blood vessels. Blockage of angiotensin receptors

directly causes vasodilation, reduces secretion of vasopressin, and reduces production and secretion of aldosterone. Angiotensin II receptor blockers are used primarily for the treatment of hypertension where the patient is intolerant of ACE inhibitor therapy primarily because of cough (Tadevosyan *et al.,* 2011). Losartan, Valsartan and Candesartan are classical examples of angiotensin II blocking drugs. Adverse effects associated with Angiotensin II blockers include first dose orthostatic hypotension, rash, diarrhoea, dyspepsia, abnormal liver function, muscle cramp, myalgia, back pain, insomnia, decreased haemoglobin levels, renal impairment, pharyngitis, and nasal congestion. Hyperkalaemia also occurs in conjunction with other factors that alter potassium homeostasis, such as renal insufficiency (Joshi *et al*., 2010).

2.4.3.3 Use of alternative medicine in management of hypertension

Traditional medicine has substantial impact on hypertension management in sub-Saharan Africa. The use of traditional medicine among adults in Sub-Saharan Africa is as high as 90%, with a prevalence range of 38.5-90% (Oreagba *et al*., 2011). Traditional herbal medicine is unremarkably used around the world for hypertension treatment and other cardiovascular diseases in general (Grant *et al*., 2012). Table 2.2 shows some of the selected medicinal plants used in management of hypertension. In Tanzania, adults admitted with hypertension complications frequently report prior traditional medicine use (Liwa *et al*., 2014). Efforts by the low-income group, especially the rural areas in the developing countries, to manage hypertension and its fatal complications in the presence of the scarce socioeconomic resources, have led more people opting for herbal medicines (Tabassum and Ahmad, 2011). However, more scientific research is required to verify the potency and elucidate the safety profile of these herbal medicines.

Table 2.2: Some of the documented medicinal plants with antihypertensive effects

Plant Name	Family	Common Name (Language)	Plant Part Used	Ethnobotanical usage in hypertension Treatment	References
Agathosma betulina	Rutaceae	Regteboegoe	Leaves, Stem	Infusion	Olorunnisola *et al.*, 2011
Acokanthera oppositifolia	Apocynaceae	Inhlungunyembe (Zulu)	Leaves, Roots Stem	Maceration	Van Wyk *et al.*, 1997
Allium sativum	Amaryllidaceae	Garlic	Bulb, Flower Bud	Chew Raw	Thring, & Weitz, 2006).
Amaranthus hybridus	Amaranthaceae	Bhoonko (Tonga)	Leaves	Maceration	Ramesar *et al.*, 2008
Bidens pilosa	Asteraceae	Beggar's Tick, Black-Jack	Leaves	Decoction	Bartolome *et al.*, 2013
Canabis sativa	Cannabaceae	Nsangu, Lubange (Tonga)	Leaves	Infusion, Decoction	De Wet *et al.*, 2016
Carpobrotus dimidiatus	Mesembryanthemaceae	Ikhambi Lamabulawo (Ndebele)	Leaves, Stem, Fruit	Decoction	De Wet *et al.*, 2016
Carpobrotus edulis	Aizoaceae	Igcukuma (Xhosa)	Leaves, Stem	Decoction	Van Wyk *et al.*, 1997; Duncan *et al.*, 1999
Conyza scabrida	Asteraceae	Umanzimnyama (Zulu)	Leaves	Infusion	Thring & Weitz, 2006).
Lantana camara	Verbenaceae	Ubukhwebezane (Zulu)	Roots	Infusion/Decoction	Semenya *et al.*, 2012
Lippia javanica	Verbanaceae	Umsuzwane (Ndebele)	Leaves	Decoction	De Wet *et al.*, 2016
Persea americana	Lauraceae	Avocado Tree	Leaf, Pulp, Fruit, Root	Decoction	Semenya *et al.*, 2012
Ricinus communis	Euphorbiaceae	Umhlakuva (Ndebele)	Leaves	Decoction	De Wet *et al.*, 2016
Tephrosia capensis	Fabaceae	Unknown	Roots	Decoction	Moffett, 2010
Tulbaghia acutiloba	Alliaceae	Ishaladi (Zulu)	Bulb, Flower, Whole Plant	Infusion, Decoction	Moffett, 2010
Turraea floribunda	Meliaceae	Umadlozane (Zulu)	Leaves, Roots	Infusions	Moffett, 2010
Vangueria infausta	Rubiaceae	Umtulwa (Ndebele)	Bark, Leaves	Maceration, Infusion	De Wet *et al.*, 2016

2.5 Pharmacological activity of *Carpobrotus edulis*

2.5.1 Antimicrobial activity of *Carpobrotus edulis*

This section covers in detail the studied antibacterial and antifungal effects of various *Carpobrotus edulis* extracts.

2.5.1.1 Antibacterial activity

The antimicrobial activity of *Carpobrotus edulis* extracts has been extensively researched. The phytochemicals have showed considerable activity against various microbes. The compounds isolated by Van der Watt and Pretorius (2001) demonstrated remarkable antibacterial activity against the gram negative *Moraxella catharalis* as well as gram positive cocci, *Staphylococcus epidermidis* and *Staphylococcus aureus*. A phenolic compound, hyperoside and a flavonone glycoside called neohesperidin also demonstrated activity against *Pseudomonas aeruginosa.* The growth of *Bacillus subtilis* and *Streptococcus pneumonia* colonies were only inhibited by a phenolic compound called ferrulic acid.

Methanol extracts of *Carpobrotus edulis* however revealed no antibacterial activity against the methicillin resistant *Staphylococcus aureus* or against the multidrug resistant *Mycobacterium tuberculosis* (Martins *et al*., 2005). These extracts however are able to impede bacterial growth once phagocytosed by monocyte derived human macrophages. Seasonal variation in the antimicrobial activity of *Carpobrotus edulis* against *Pseudomonas aeruginosa, Enterococcus feacalis, Escherichia coli* and *Staphylococcus aureus* was also evaluated (Chokoe *et al*., 2008). The minimum inhibitory concentration values for the spring extracts were lower than those of the autumn extracts suggesting that the spring extracts were more effective against all the test organisms. When the total activity was taken into account, the autumn extracts however revealed higher efficacy than the spring extracts. *Carpobrotus edulis* aqueous leaf extract demonstrated

noteworthy antibacterial activity against *Mycobacterium aurum*. The ethanolic extract showed significant activity against *staphylococcus aureus, Bacillus cereus, S* and *Mycobacerium aurum* but showed weak activity against *Klebsiella pnuemoniae* and Escherichia *coli* (Buwa & Afolayan, 2009). Among the solvents evaluated, the ethanolic extract showed the weakest antibacterial activity in comparison to both the dichloromethane and water extracts. Meddeb *et al.,* (2017) also confirmed the reports that *Carpobrotus edulis* leaf extracts has high antibacterial properties, particularly against the Gram positive *Staphylococcus aureus* and *Bacillus cereus* strains.

Martins *et al*., (2011) isolated numerous compounds from *Carpobrotus edulis* and evaluated them for antibacterial activity against multidrug-resistant (MDR) bacteria. Oleanolic acid, a pentacyclic triterpenoid demonstrated strong activity against several bacterial strains. Another pentacyclic triterpene, Uvaol displayed the most effective modulation of efflux activity by multidrug-resistant Gram-positive strains. The activities of numerous compounds isolated from *Carpobrotus edulis* were evaluated against multidrug-resistant (MDR) bacteria (Martins *et al.,* 2011). Oleanolic acid displayed good antibacterial activity against several bacterial strains with uvaol displaying the most effective modulation of efflux activity by MDR Gram positive strains. *Carpobrotus edulis* found on the Tunisian coast also displayed notable antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* (Ibtissem *et al.,* 2012).

2.5.1.2 Antifungal activity

Essential oils were extracted from fresh leaves of *Carpobrotus edulis* for antifungal activity evaluation. Four solvents; hexane, acetone, water and ethanol were also used to extract fresh *Carpobrotus edulis* leaves that were also tested for antifungal activity. The essential oils proved

to be more effective in inhibiting fungal growth compared to extracts from the four listed solvents. These essential oil extracts revealed antifungal activity against *Candida krusei, Candida albicans, Candida glabrata Candida rugosa,* and *Cryptococcus neoformans* with minimum inhibitory concentration ranges of 0.02- 0.31 mg/ml (Omoruyi *et al.,* 2014). The antifungal activity of the isolated essential oils was comparable to standard antifungal agents, nystatin and amphotericin B which were used as controls in the experiment. Hexane extracts were also effective against all the five fungal isolates while acetone extracts were only effective against *C. krusei* at 0.04 mg/ml. The results are consistent with those of Wilfred *et al*., (2011) when the effects of the acetone extracts of *arctotis arctotoides* on the growth of some opportunistic fungi associated with HIV/AIDS were evaluated. Ethanol and aqueous extracts had no considerable antifungal activity. Aqueous extracts could not inhibit the growth of the five fungi isolates, even at the highest concentration of 5 mg/ml (Omoruyi *et al*., 2014).

2.5.2 Antioxidant activity

Chokoe *et al.,* (2008) evaluated the seasonal variation in the antioxidant activity of *Carpobrotus edulis* extracts. The ethyl acetate, acetone and methanol extract reportedly had an antioxidant compound which was more evident in the autumn extracts. The antioxidant activity of *Carpobrotus edulis* growing in the Tunisian coast was also evaluated (Ibtissem *et al.,* 2012). A higher *Carpobrotus edulis* antioxidant activity, concentration of up to 2 mg/ml, in the DPPH assay compared to *Mesembryanthemum crystallinum* was reported (Ibtissem *et al*., 2012). A higher proportion of flavonoids and phenols may be responsible for such an outstanding antioxidant activity. *Carpobrotus edulis* had even a higher antioxidant activity than that of butylated hydroxyanisole, a synthetic antioxidant. The antioxidant activity of *Carpobrotus edulis*

was also evaluated by Omoruyi *et al.*, (2012) and found out that the ethanol and aqueous extracts demonstrated the best antioxidant activity.

Falleh *et al.*, (2011a) extensively evaluated the antioxidant activity of *Carpobrotus edulis*. The antioxidant properties and phenolic compounds of *Carpobrotus edulis* were characterized in the root, stem and the leaf. The aerial parts of the plants were reported to have higher antioxidant activity than the roots. The aerial parts had the highest polyphenolic content compared to the roots, explaining the higher antioxidant activity. All studied organs had a significantly higher activity of butylated hydroxytoluene, with maximal efficiency for stems followed by leaves then roots. In the characterization of polyphenols responsible for the strong antioxidant properties of *Carpobrotus edulis* using LC/ESI-MS/MS, the methanol extract from the leaf, root and stem showed the highest scavenging activity against ABTS and DPPH radicals (Falleh *et al*., 2011b). The leaf extract contained mainly procyanidins and the stem extracts mostly had propelargonidins responsible for the potent antioxidant activity. Despite methanol extracts being richer in total polyphenol content compared to the ethanol, the latter had higher antioxidant activity than the former.

2.5.3 Antiproliferative activity of *Carpobrotus edulis*

Carpobrotus edulis extracts are purportedly reported to have antiproliferative activity. Compounds isolated from the *Carpobrotus edulis* leaf extracts using methanol water and hexane were evaluated for their antiproliferative effects on mouse lymphoma parental cells and human MDR1-transfected mouse lymphoma cells. All the compounds isolated reduced the proliferation of both cell lines. Catechin, Oleanolic acid and Uvaol were some of the isolated compounds and their antiproliferative effect was more significant in the parental cell lines. The multidrug

resistant cell line was sensitive to epicatechin and monogalactosydiacylglycerol (MDGD) (Martins *et al*., 2010). In all the isolated compounds, Uvaol had the most efficacious antiproliferative activity and is a potential lead in the reversal of multidrug resistance. Alkaloids from the family Aizoaceae have purported anticancer activity, even though the species of this family have invited minimal attention. Ordway *et al*., (2003) revealed that *C. edulis* extract is non-toxic at concentrations that inhibit a verapamil sensitive efflux pump of L5178 mouse T cell lymphoma cell line thereby making these multidrug resistant cells sensitive to anticancer drugs. Hydroethanolic and aqueous extracts of *C. edulis* were also reported to have cytotoxic effects against HCT116 cells, a human colon cancer cell line (Hafsa *et al*., 2016). The ethanol-water extracts were more effective with substantial reduction in cell viability after 24 hours of incubation.

2.5.4 Neurological activity of *Carpobrotus edulis*

The results from Custódio *et al*., (2012) reveal that *C. edulis* has anticholinesterase activity against acetylcholinesterase and butyrylcholinesterase. *Carpobrotus edulis* is thus considered a potential lead in future research and alternative therapy for the management of neurological conditions associated with decreased acetylcholine levels in the brain.

2.6 Phytochemicals and their analysis

2.6.1 Sources and Classification

Phytochemicals are biologically active chemical compounds naturally found in plants. These bioactives defend plants from disease and damage and they give plants their aroma, colour and flavour (Valevan, 2015). These bioactive constituents of plants are steroids, carotenoids, terpenoids, flavanoids, tannins, saponins, alkaloids and glycosides. These phytochemicals have

various characteristics which are beneficial to human health, for example antimicrobial activities, anticancer activities and antihypertensive activities.

2.6.1.1 Alkaloids

Alkaloids are one of the largest groups of plant secondary metabolites, being present in several economically relevant plant families. Alkaloids encompass neuroactive molecules, such as caffeine and nicotine, as well as life-saving medicines including emetine used to fight oral intoxication and the antitumor drugs vincristine and vinblastine (Matsuura & Fett-Neto, 2017). Alkaloids can act as defence compounds in plants, being efficient against pathogens and predators due to their toxicity. Toxic effects, in general, depend on specific dosage, exposure time, and individual characteristics, such as sensitivity, site of action, and developmental stage (Bribi *et al.*, 2015). Toxicity effects of alkaloids can be both harmful and beneficial depending on the ecological or pharmacological context.

2.6.1.2 Tannins

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. Tannins have a various physiological roles which include chelation of transition metals, inhibition of pro-oxidative enzymes and lipid peroxidation. Tannins are also known to inhibit tumour growth and mutagenicity of carcinogens (Ngoci *et al.*, 2011). The anticarcinogenic and antimutagenic potentials of tannins may be related to their antoxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation.

2.6.1.3 Flavonoids

Flavonoids are phenolic water soluble structural derivatives of flavones, containing conjugated aromatic systems, often bound to sugars as glycosides. Flavonoids are well known for their beneficial effects on health. They are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. Flavonoids such as quercetin, act as chain breaking anti-oxidants, and by preventing oxidation of low-density lipoprotein by macrophages and metal ions like copper (Santos *et al.,* 2017).

2.6.1.4 Saponins

Saponins are a diverse group of compounds widely distributed in the plant kingdom, which are characterized by their structure containing a triterpene or steroid aglycone and one or more sugar chains. Saponins have soap-like properties and can be detected by their ability to cause foaming and to haemolyse blood cells (Güçlü-Üstündağ & Mazza, 2007). The biological roles of saponins include antiprotozoal activity, antibacterial activity and boosting respiratory system as expectorant.

2.6.1.5 Phytosteroids

Phytosteroids are steroidal compounds of plant origin which have estrogenic effects and can act as agonists, antagonists, or have a mixed agonistic/antagonistic activity to animal steroidal receptors (Di Gioia & Petropoulos, 2019). They are mainly used to treat reproductive complications such as treatment of venereal diseases. They are also used during pregnancy to ensure an easy delivery, as well as to promote fertility in women and libido in men.

2.6.1.6 Terpenoids

Terpenoids are derivatives of isoprene molecule and hence commonly referred to as isoprenoids. They form the largest and most diverse class of phytochemicals among the different compounds

produced by plants. Traditionally, plant-based terpenoids have been used by humans in the food, pharmaceutical, and chemical industries, and more recently have been exploited in the development of biofuel products (Tholl, 2015). Terpenoids have proved to have antimicrobial activities as well as antineoplastic activities.

2.6.1.7 Cardiac Glycosides

Glycosides are compounds containing a carbohydrate and a non-carbohydrate residue in the same molecule. Cardiac glycosides occur as a complex mixture together in the same plant and most of them are toxic, however many have pharmacological activity especially to the heart (Morsy, 2017). They are used in treatment of congestive heart failure, whereby they inhibit sodium-potassium ATPase pump that causes positive ionotropic effects and electrophysiological changes. This strengthens heart muscle and the power of systolic concentration against congestive heart failure.

2.6.1.8 Phenols

Phenols form one of the largest phytochemical groups, varying in size from a simple structure to complex ones such as lignins. Phenols contribute to the plant's colour, flavour and astringency. They have various known physiological roles which include anti-carcinogenic activities, analgesic activities, anti-mutagenic and anti-oxidation activities (Kougan *et al.*, 2013).

2.6.1.9 Anthraquinones

Anthraquinones are a group of secondary metabolites structurally related to anthracene and their glycosides (Chien *et al.*, 2015). These compounds impart colour to plants and have been widely utilized as natural dyes. Anthraquinones are not strictly quinones, some of them like sennosides, are diathrones (Simpson and Amos, 2017). Anthraquinones have varied physiological properties

which include antifungal and antiviral activities. Anthraquinones are extracted commercially from plants for use as irritant cathartics.

2.6.2 Phytochemical screening of *Carpobrotus edulis*

Phytochemical analysis may be both qualitative (screening) or quantitative. Phytochemical screening defines a series of tests which determines only the presence or absence of certain chemical substances in a plant. Standard methods have been devised to qualitatively detect the presence or absence of different phytochemicals. Preliminary screening is crucial in the detection of the phytochemicals present in medicinal plants (Ajuru *et al.*, 2017). This will eventually lead to further quantitative analysis and identification of these phytochemicals, a critical step in drug discovery and development.

Eman (2011) performed the phytochemical screening of various succulent plants found in Egypt and *Carpobrotus edulis* was one of them. *Carpobrotus edulis* flowers were found to be the richest organ containing the highest amounts of all the measured phytochemicals except the leaf which had higher levels of tannins, anthraquinones and sulphates than the flowers (Table 2.3).

The stems were found to be rich in polyphenols and contained the highest total flavonoid content. As per the phytochemical screening findings of Eman (2011), Van der Watt and Pretorius (2001) reported that the leaves of *C. edulis* had high tannin content. Qualitative phytochemical screening of the *C. edulis* leaf extracts revealed the presence of secondary metabolites in aqueous, ethanol, acetone and hexane extracts.

Table 2.3: **Phytochemical screening of *Carpobrotus edulis* plant parts from Egypt**

Phytochemical group	Levels of phytochemicals in different plant parts		
	Stems	**Leaves**	**Flowers**
Saponins	++	+	++
Chlorides	+	+	+
Sulphates	+	++	+
Coumarins	+	+	+
Flavonoids	+	++	+++
Alkaloids	+	+	++
Anthraquinones	++	++	+
Irodoids	-	-	-
Cyanogenic glycosides	+	+	+
Cardiac glycosides	++++	+++	++++
Carbohydrates and / or Glycosides	+	+	+
Unsaturated sterols and / or Triterpenoids	+	+	+
Tannins	++	+++	+++

Very highly present++++, highly present+++, moderately present ++, lowly present, not detected –

Source: Eman, 2011

2.6.3 Quantitative phytochemical analysis of *Carpobrotus edulis*

Quantitative analysis entails finding the amounts of different phytochemical in a specific part of a plant. There are also different standardised methods employed in order to quantify different types of phytochemicals. The quantification is very important. Quantification of phytochemicals helps to purify and identify the phytochemical compounds (Ganga *et al.*, 2017). The total polyphenol content found within the leaves of *C. edulis* varied significantly between other plant parts. Quantitatively, the leaf extract showed a significantly higher concentration of phenolic compounds compared to the stems and especially the roots (Faller, *et al*., 2011a).

There is no particular solvent that is known to extract all the compounds on its own from the plant because of the huge differences in the nature of phytochemical constituents found in a plant. Four solvents hexane, ethanol, acetone and water were used to extract *C. edulis* leaves to accommodate the range of polarities of the compounds present. The extracts were quantitatively

analysed for phytochemicals. The acetone extracts had a high percentage of phenolics and a considerable amount of alkaloids and proanthocyanidins in the aqueous extract (Omoruyi *et al*., 2012); (Table 2.4). Tannins and saponins were major constituents in the ethanol extract and flavonoids and flavonols were at a higher concentration in the hexane extract (Table 2.5).

Table 2.4: **Phytochemical screening of the extracts from *Carpobrotus edulis* leaf**

Phytochemicals	**Aqueous**	**Ethanol**	**Acetone**	**Hexane**
Phenolics	+++	+++	+++	++
Flavonoids	+	+	+	+
flavonols	+	+	+	+
Proanthocyanidins	+++	+++	+++	+++
Tannins	+++	+++	+++	++
saponins	++	++	++	+
alkaloids	++	++	++	+

Highly present+++, moderately present -++, lowly present +

Source: Omoruyi *et al.,* 2012

Table 2.5: Quantitative analysis of the phytochemical evaluated from the leaf of *C. edulis*

Phytochemicals	**Amount of phytochemical compounds (mg/g)**			
	Aqueous	**Ethanol**	**Acetone**	**Hexane**
Phenols	517.71±0.40	330.87±0.04	557±0.23	64.14±0.15
Flavonoids	0.29±0.01	0.28±0.01	0.65±0.04	1.19±0.04
Flavonols	0.05±0.001	0.05±0.001	0.23±0.05	0.19±0.03
Proanthocyanidins	896.7±0.05	115.28±0.007	753.87±0.02	134.91±0.01
Tannins	461±0.07	489±0.28	384±0.14	64±0.14
Saponins	34±0.21	45±0.26	11±0.071	2±0.035
Alkaloides	45±0.06	38±0.02	31±0.021	3±0.014

Source: Omoruyi *et al.,* 2012

In Comparison to *Mesembryanthemum crystallinum* , a plant in the same family with *Carpobrotus edulis,* the determination of the flavonoids in the plant extracts revealed a higher content in in *C. edulis* (116.16 ± 3.34 µg/mg) in comparison with *M. crystallinum* (4.85 ± 0.9 µg/mg). *C. edulis* extract (104.69 ± 0.48 µg/mg) also had higher phenol content than *M. crystallinum* (23.89 ± 0.27 µg/mg) (Ibtissem *et al.,* 2012). Seasonal variation in the phytochemical composition of *Carpobrotus edulis* extracts were evaluated by Chokoe *et al*., (2008). The prevalence of phytochemicals within the autumn leaf debris samples, regardless of the extracting solvent used, suggests that there is a higher concentration of phytochemicals within the leaf tissue of the plant during autumn and less of them are being circulated around the plant.

2.6.4 Phytochemical identification

Martins *et al*., (2011) used a 1D, 2D NMR and MS investigations to identify compounds known as triterpens (β-amyrin, uvaol and oleanolic acid), monogalactosyldiacylglycerol, catechin, epicatechin and procyanidin B5 from methanol *C. edulis* extracts. Phenolic composition was also analysed and revealed the presence of sinapic acid, luteolin7-o- glucoside, hyperoside, ferrulic acid isorhamnetin-o- rutinoside, allergic acid and isoquercitrin from hydroethanolic and aqueous extracts of *C. edulis* (Van der Watt and Pretorius 2001; Meddeb *et al*., 2017). Omoruyi *et al*., (2014) using the GC-MS analysis investigated the chemical composition of *C. edulis* in hexane, acetone and ethanol. The identified phyto-constituents are displayed in Tables 2.6, 2.7 and 2.8.

Table 2.6: **Phyto-constituents identified in the hexane extract of *C. edulis*.**

Retention time	compounds	Formula
3.9	2-Pentadecanone, 6,10,4- trimethyl	$C_{18}H_{36}O$
4.5	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$
5	Heptacosane	$C_{27}H_{56}$
5.3	1-Heptatriacotanol	$C_{37}H_{76}O$
5.54	n-Octyl-5-oxoheptadecanamide	$C_{25}H_{49}NO_2$
5.75	Dodecanoic acid	$C_{12}H_{24}O_2$
7.78	Phytol	$C_{20}H_{40}O$
8.6	Dibutyl phthalate	$C_{16}H_{22}O_4$
8.67	n-hexadecanoic acid	$C_{14}H_{28}O_2$
8.93	2-tertbutyle cyclohexylpropylphosphonofluoridate	$C_{13}H_{26}FO_2P$
10.55	2-Pyrrolidinone, 1-(9-octadecenyl)	$C_{22}H_{41}NO$
11.47	Pyrrolidine, 1-(1-oxo-7,10-hexadecadienyl)	$C_{20}H_{35}NO$
13.96	Nonacosane	$C_{29}H_{60}$
4.331	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$
18.09	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl	$C_{30}H_{50}$
19	Octadecanoic acid	$C_{18}H_{36}O_2$
19.87	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$
8.6	Tetradecanoic acid	$C_{14}H_{28}O_2$
20.6	Tetratriacontane	$C_{34}H_{70}$
21.62	9,12-Octadecadienoic acid (Z,Z)-2 3-dihydroxypropyl ester	$C_{18}H_{32}O_2$
24.07	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)	$C_{21}H_{36}O_4$
27.08	Eicosanoic acid	$C_{20}H_{40}O_2$
32.4	α-Amyrin	$C_{30}H_{50}O$
34.87	1-Heptatriacotanol	$C_{37}H_{76}O$
40.57	9,19-Cyclolanost-24-en-3-ol, acetate, (3β)	$C_{32}H_{52}O_2$
48.09	Lupeol	$C_{30}H_{50}O$
56.05	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1Hcyclopenta[a]phenanthren-3-ol	$C_{27}H_{46}O$
57.35	Vitamin E	$C_{29}H_{50}O_2$
58.06	17-(1,5-Dimethylhexyl)-2,3-dihydroxy-10,13-dimethyl-1,2,3,7,8,9,10,11,12,13,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-6-one	$C_{27}H_{44}O_3$
59.83	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	$C_{30}H_{48}O$

Source: Omoruyi *et al.*, 2014

Table 2.7: Phyto-constituents found in the acetone extract of *C. edulis.*

Retention time	Compounds	Formula
4.51	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$
5.113	6,6-Dimethyl-10-methylene-1-oxa-spirodecane	$C_{12}H_{20}O$
5.3	1-Heptatriacotanol	$C_{37}H_{76}O$
6.09	Dodecanoic acid	$C_{12}H_{24}O_2$
6.286	17-Pentatriacontene	$C_{35}H_70$
7.78	Phytol	$C_{20}H_{40}O$
8.6	Tetradecanoic acid	$C_{14}H_{28}O_2$
8.67	n-Hexadecanoic acid (dibutyl ester)	$C_{14}H_{28}O_2$
12.86	n-Hexadecanoic acid (bis-2-ethylhexyl ester)	$C_{16}H_{32}O_2$
13.96	Nonacosane	$C_{29}H_60$
43.5	α-Amyrin	$C_{30}H_{50}O$
48.09	Lupeol	$C_{30}H_{50}O$

Source: Omoruyi *et al.*, 2014

Table 2.8: Phyto-constituents found in the ethanol extract of *C. edulis.*

Retention time	Compounds	Formula
32.4	β-Amyrin	$C_{30}H_{50}O$
42.918	α-Amyrin	$C_{30}H_{50}O$
48.09	Lupeol	$C_{30}H_{50}O$

Source: Omoruyi *et al.*, 2014

2.7 Toxicological studies

Toxicological studies are experiments done to determine hazardous effects of a substance that may arise due to physicochemical interaction with living tissue. They can be classified based on the duration of the study into acute, subacute and chronic toxicity.

2.7.1 Acute toxicity

Acute toxicity is caused by an agent when it is administered in one or more doses over a period not exceeding 24 hour and involves harmful effects to the organism through a single or short term exposure**.** Acute toxicity studies have also been used during the selection of starting doses for phase-I human and animal studies, and provide information relevant to acute overdosing in humans and animals (Diallo *et al*., 2010). The acute toxic class method, a step-wise procedure, involves the use of three animals of a single sex per step (Parasuraman, 2011). Depending on the mortality and/or moribund status of the animals, on average 2 to 4 steps may be necessary to allow judgment on the acute toxicity of the substance.

2.7.2 Subacute Toxicity

The sub-acute toxicity test in the study was based on OECD guideline 407 (2008), repeated dose 28-day oral toxicity study in rodents. In this form of toxicity, adverse effects occur as a result of repeated daily dosing of a chemical or exposure to the chemical, for part of an organism's lifespan usually not exceeding 10 % of the animals' lifespan. Exposure for 28 days provides a first-hand indicator of potential subacute toxicity. The test is intended to investigate effects on a very broad variety of potential targets of toxicity. It provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time, including effects on nervous, immune and endocrine systems (Parasuraman, 2011). The duration of exposure is normally 28 days in rodents where results are used for hazard identification and risk

assessment (OECD, 2008). All the knowledge gathered from the studies is used in selecting doses for repeat-dose studies as a source of preliminary identification of target organs of toxicity, and may also reveal delayed toxicity. Sub-acute toxicity studies in animals are essential for any pharmaceutical products especially those intended for human use.

2.7.3 Chronic toxicity

Chronic studies are similar to subacute toxicity studies, except that in chronic toxicity studies the timeframe of exposure is prolonged. They are performed in order to evaluate the cumulative long term toxicity of substances. Chronic toxicity studies detect the general toxic effects, including physiological, neurological, hematological, biochemical, and histopathological effects (Gadaga & Tagwireyi, 2014). These studies are usually done with rodent species, mice and rats.

2.7.4 Genotoxicity and Mutagenicity Testing

Studies have revealed that traditional medical plants may have in vitro mutagenic and/or carcinogenic properties. Traditional medicinal plants should be screened for their genotoxicity potential. These plants may have been in use for ages and therefore deemed safe, yet the genotoxicity effects may not appear immediately but over a long period of time (Ganga *et al*., 2017). Ames test and alkaline comet assay are standard procedures developed to assess the mutagenicity of extracts.

2.7.5 Toxicology of *Carpobrotus edulis*

A selected number of *Carpobrotus* species with medicinal properties were tested for cytotoxicity using the brine shrimp lethality test. The aqueous extract of *Carpobrotus mellei* and tthe methanol extract of *Carpobrotus quadrifidus* showed the highest activity than *Carpobrotus edulis* and other species tested (Jooste, 2012). Akhalwaya *et al.,* (2018) investigated the cytotoxicity of indigenous South African medicinal plants used to treat oral infections.

Carpobrotus edulis is one of the medicinal plants tested and was considered non- toxic with percentage mortality rate of 47.43% at 24 hour and 48.06% at 48 hours. Cock and Van Vuuren (2014) also found out that aqueous and methanol extracts of *C. edulis* are either non-toxic, or of low toxicity in the brine shrimp lethality bioassay.

Dugesia sicula Lepori, 1948, a freshwater planarian was used in order to investigate the effect of aqueous-acetone *C. edulis* extracts on regeneration. Morphological changes were evident on microscopic analysis of *Dugesia sicula Lepori* in ordinary medium containing phenolic extracts at non-toxic concentrations. The study suggested that *C. edulis* polyphenols can have harmful effects on the development of stem cells (Meddeb *et al*., 2017). *Carpobrotus edulis* polyphenols can therefore have ecotoxicological impact on the planarians' physiology in the environment.

2.8 Knowledge gap

The literature review shows that *Carpobrotus edulis* is a common medicinal plant used to treat hypertension in Southern Africa (Omoruyi *et al.,* 2012). Traditional healers in the Eastern Cape use the juice and sap from *Carpobrotus edulis* leaves to treat hypertension. *Carpobrotus edulis* have proved to be important in the treatment of chronic non-communicable diseases like hypertension and diabetes mellitus (Rocha *et al*., 2017; Davids *et al*., 2016; Al-Faris *et al.,* 2010). However there is paucity of information with regard to the scientific validation on medicinal use of *Carpobrotus edulis* in hypertension treatment. Despite the extensive literature on the phytochemicals of *Carpobrotus edulis*, the phytochemical content of *Carpobrotus edulis* plants growing in Zimbabwe remains unknown. Scientific data regarding safety evaluation of *Carpobrotus edulis* extracts is also scanty. Phytotherapeutic products are usually mistakenly regarded as non-toxic because they are natural. However these products contain bioactive

principles which can potentially cause adverse effects. It is therefore of paramount importance to perform a toxicological safety investigation of *Carpobrotus edulis*.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter covers the materials and methods used in Phytochemical screening, Toxicological studies and determination of antihypertensive efficacy of *Carpobrotus edulis.*

3.2 Study Area

The study was carried out in the Pharmacology and Toxicology Laboratory at the University of Zimbabwe in Mount Pleasant, Harare Province in Zimbabwe (Figure 3.1). Mount Pleasant is a residential low density suburb located in the northern part of Harare. Mount Pleasant has an altitude of about 1 483 metres above sea level. It has a warm temperate climate. There are three main seasons; a warm, wet season from November to March/April; a cool, dry season from May to August; and a hot, dry season in September/October. The average maximum daytime temperature in January is 25°C, dropping to an average maximum of around 20°C in July. The average annual rainfall is about 855 mm. Mount Pleasant is bordered by five suburbs namely Vainona, Emerald Hill, Belgravia, Borrowdale west and Marlborough. In Mount Pleasant, *Carpobrotus edulis* is not found in its natural environment but it is propagated in the individual gardens for ornamental purposes. *Carpobrotus edulis* was collected from four sites in Mount Pleasant and the GPS coordinates for these collection sites are S 17˚470148 E031˚ 46.926, S17˚46.234 E031˚031 03.110, S17˚46.728 E031˚03.008 and S17˚46.786 E031˚03.238.

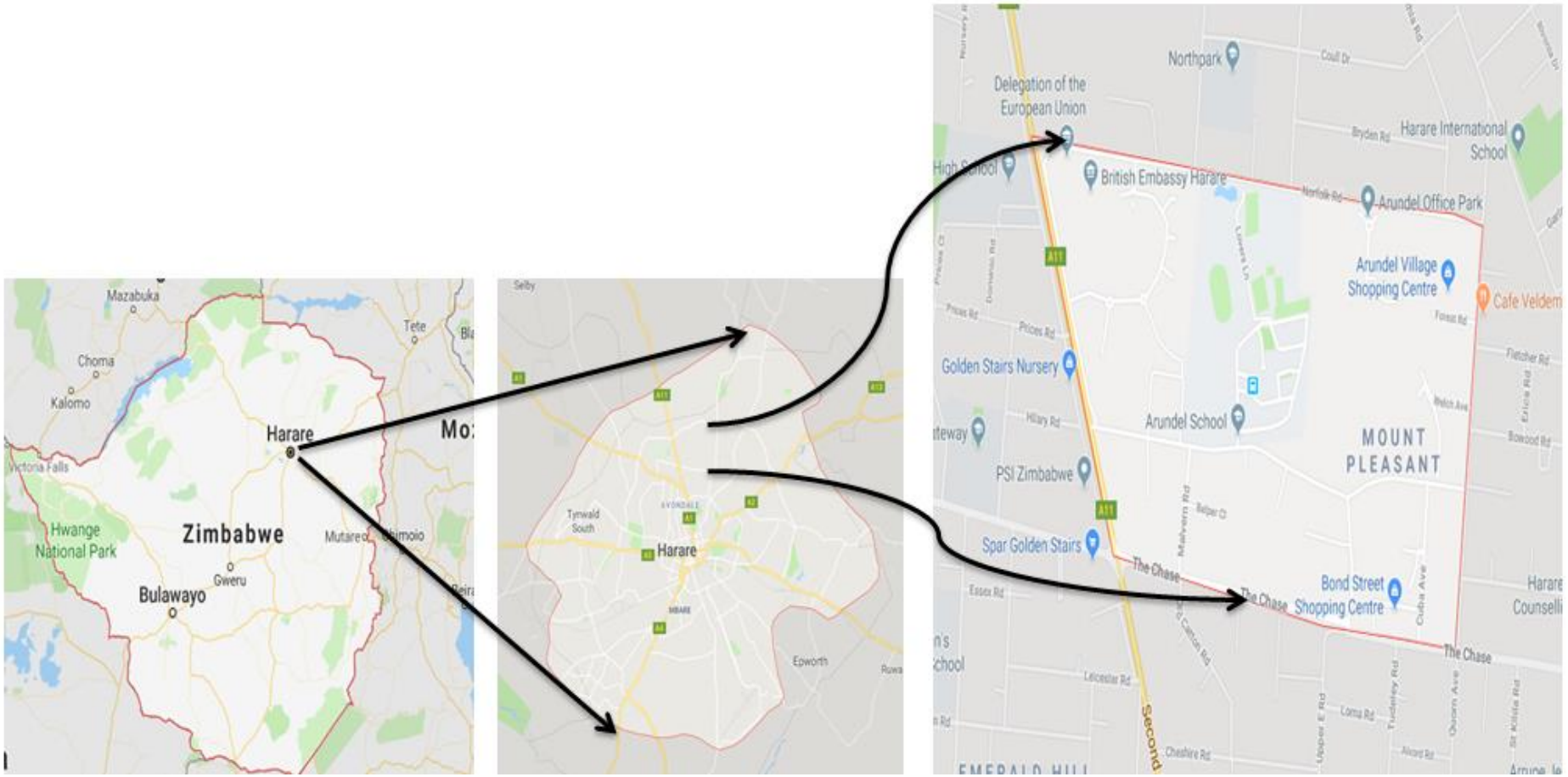

Figure 3.1: Shows the map of Mount Pleasant and its location in Harare, Zimbabwe.

3.3 Phytochemical Screening of *Carpobrotus edulis*

3.3.1 Collection and identification of the plant

Eight (8) kilograms of *Carpobrotus edulis* plant leaves were collected at four sites mentioned in section 3.2. These collection sites were chosen because of convenience; they are nearer to the University of Zimbabwe where the study was conducted. The plant was authenticated by a Botanist from the National Herbarium and Botanical Gardens of Zimbabwe, and a voucher specimen was properly marked and stored. The voucher number of the voucher specimen is 'Mudimba, T.1 08/01/2019'.

Figure 3.2: Collection of *Carpobrotus edulis* in a botanical garden along Cannock Road, Mount pleasant (Picture taken by Toonse N. Mudimba)

3.3.2 Preparation of plant material

The leaves were spread thinly on top of the table in a well-ventilated, rodent and dust free room. The leaves were air dried at room temperature (22 ^{o}C ±3 ^{o}C) for 12 days and ground into powder using a laboratory pulveriser (Mill 1600). The powder weighing 200 g, weighed using a Compact electronic Scale (SF-400A, 100G/0.01g) was macerated in distilled water at a ratio of 1 to 6 (w/v) in a volumetric flask. The suspension was macerated for 48 hours at room temperature with constant shaking. The suspension was then filtered using Whatman® filter paper (number 4). The filtrate was freeze dried (Edwards-Freeze Dryer Modulyo, EF4) for 48 hours and the extract was weighed in order to determine yield (Awounfack *et al.,* 2016).

Calculation of percentage yield;

Percentage of crude extract yield = $(M_1/M_0) \times 100$,

Where;

M_1= Mass of extract

M_0= Mass of initial leaf powder sample.

The percentage yield of the aqueous extract of *Carpobrotus edulis* leaves was:

21.41 g/200 g X 100

= 10.71%.

The obtained aqueous extract was a semi solid mass, light brown in colour

This product was then stored under refrigeration (4 ˚C) in well closed, light resistant bottles while awaiting phytochemical analysis and administration to experimental animals.

3.3.3 Phytochemical screening

Qualitative phytochemical screening methods were used in order to identify the phytochemical constituents of aqueous leaf extracts of *Carpobrotus edulis*. The standard qualitative methods used by Ajuru et al., (2017) were used in order to detect the presence or absence of phenols, flavonoids, anthraquinones, alkaloids, terpenoids, saponins, tannins and glycosides.

3.3.3.1 Ferric chloride test for phenols

Approximately 0.5 grams of *C. edulis* extract were mixed with 10 ml of distilled water and then filtered. Five drops of five (5%) ferric chloride solution were added to 2 ml of the filtrate. Appearance of green, blue-green or blue-black precipitate was considered positive for phenols.

3.3.3.2 Alkaline reagent test for Flavonoids

Five drops of five percent (5%) sodium hydroxide solution were added to one millilitre of *C. edulis* extract. Two millilitres of hydrochloric acid were then added. Intense yellow coloration which disappeared on addition of hydrochloric acid was considered positive.

3.3.3.3 Borntragger's test for anthraquinones

Ten millilitres of benzene were added to five milligrams of *C. edulis* extract; the resulting mixture was shaken and then filtered. Five millilitres of 10% ammonia solution were added to the filtrate and then agitated. Pink, red or violet colour in the lower phase was considered positive.

3.3.3.4 Test for alkaloids

One gram of *C. edulis* extract was mixed with 5 ml of 1% aqueous hydrochloric acid in a water bath and then filtered. Two millilitres of this filtrate were collected in a test tube and one millilitre of dragendorrf's reagent was added along the inner wall of the test vessel. A reddish brown precipitate was considered positive.

3.3.3.5 Liebermann-Burchard test for terpenoids

Two millilitres of acetic acid was added to 0.2 grams of *C. edulis* extract. The solution was cooled well in a freezer then concentrated sulfuric acid was added cautiously. Colour change from violet to blue or bluish green was considered positive.

3.3.3.6 Foam test for saponins

One gram of *C. edulis* extract was boiled with 5 ml of distilled water and then filtered. To the filtrate, approximately 3 ml of distilled water was added and shaken vigorously for about 5 minutes. Persistence frothing was considered positive.

3.3.3.7 Test for tannins

Screening for tannins was done using both ferric chloride and lead acetate tests. For the ferric chloride test, half a gram (0.5 g) of the *C. edulis* extract was dissolved in 2 ml of distilled water and filtered. Two drops of ferric chloride was then added to the filtrate. Development of a blue-black precipitate indicated the presence of tannin. For the lead acetate test, in a test tube containing about 5 mg of *C. edulis* extract, a few drops of 1% solution of lead acetate were added and the formation of a yellow or red precipitate indicated the presence of tannins.

3.3.3.8 Test for Glycosides

Carpobrotus edulis extract weighing about 2 mg was dissolved in 1 ml distilled water and then aqueous sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

3.4 Toxicological studies of aqueous extracts of *Carpobrotus edulis*

3.4.1 Ethical considerations

Before commencement of the study, approval on the ethical use and care of laboratory animals was obtained from two authorizing boards. Initially ethical approval (Ref number 001/2019) was granted by the Animal Research Ethics and Animal Welfare Sub-committee in the Department of Livestock and Veterinary Services of Zimbabwe. The second approval was obtained from the Biosafety, Animal Use and Ethics Committee of the Faculty of Veterinary Medicine, University of Nairobi (REF:FVM BAUEC/2019/226). The rats were disposed according to the guidelines given by the authorised ethical Committees.

3.4.2 Experimental Animals

Six week old Sprague Dawley rats of both sexes were obtained from the University of Zimbabwe Animal House and were housed in the animal holding facilities at the Faculty of Veterinary Science, University of Zimbabwe. They were caged in pairs and maintained under standard environmental conditions of 12 hours light and 12 hours darkness at 22^{o}C (±3^{o}C). The rats were fed on commercial rat pellets obtained from the Zimbabwe National foods. Drinking water was provided *ad libitum* from the tap water.

3.4.3 Acute toxicity testing

The extract was prepared as described in section 3.2.2. The acute toxicity study protocol was conducted according to the OECD test guideline 423. Three healthy female rats were used per step at any of the four fixed dose levels of 300, 600, 1200 and 2000 mg/Kg body weight. Food was withheld overnight but water was provided *ad libitum*. The Sprague Dawley rats were weighed using the electronic compact scale (SF-400A) just before extract administration through oral gavage. Food was withheld for a further 3-4 hours after the extract was administered.

Clinical observations were made for signs of toxicity focusing on respiratory, circulatory, autonomic nervous system, central nervous system, changes in mucous membranes, skin and fur, eyes, behavioural pattern and death. Animals were kept for 14 days and were weighed weekly. On the 14^{th} day, the animals were euthanized using halothane and gross necropsy was performed on all animals.

3.4.4 Sub-acute toxicity

The 28-day sub-acute toxicity study protocol was carried out as per the OECD number 408 guideline using 32 rats (16 males and 16 females) per step at any of the defined dose levels. The animals were allocated randomly into four groups of eight rats (four females and four males). Group A received 100 mg/Kg of the extract while Group B and Group C received 300 mg/Kg and 1000 mg/Kg of the extract respectively. Group D served as a normal control and rats received only distilled water .All the experimental animals in all the groups received the extract orally for 28 days.

3.4.4.1 Clinical observations

During the entire dosing period, clinical observations were made on all experimental animals for signs of toxicity focusing on respiratory, circulatory, autonomic nervous system, central nervous system, changes in mucous membranes, skin and fur, eyes, behavioural pattern and death. The rats were weighed prior to dosing at weekly intervals and the weight of each rat recorded separately. Feed and water consumption were also measured on a weekly basis.

3.4.4.2 Collection and handling of blood samples

After the treatment period, the rats were fasted overnight and put under general anaesthesia using chloroform. Blood was collected from all animals through cardiac puncture. The collected blood was divided into two portions; one for haematological analysis, which was collected in

Ethylenediaminetetraacetic acid (EDTA) tubes, and the other for biochemical analysis, collected in plain tubes. Blood for biochemical tests was centrifuged using a Hermle Centrifuge (Hermle Z206A) at 3000 revolutions per minute in order to obtain serum. The serum obtained was put in Eppendorf tubes and stored at -20^0C while awaiting biochemical analysis.

3.4.4.3 Determination of Haematological parameters

Blood collected in EDTA containing tubes was analysed in the Clinical Studies Department of the University of Zimbabwe by using a Mindray Haematology analyser (BC2800 vet). The parameters which were determined included Total Red Blood Cell count (RBC), Red Blood Cell Distribution Width (RDW), Total Leucocyte Count (WBC), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet counts and Mean Platelet Volume (MPV).

3.4.4.4 Determination of Biochemical parameters

Serum was also analysed at the Clinical Studies Department, University of Zimbabwe for biochemical parameters using a Mindray Chemical analyser (BS 120). The parameters which were determined included Alanine Aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Albumin, Total protein, Blood Urea Nitrogen (BUN) and Creatinine.

3.4.4.5 Histopathological examination

The animals were then euthanized humanely using chloroform and subjected to post-mortem examination. Internal organs were examined for gross pathological changes. The organ weights of the heart, liver and kidney were taken for every experimental animal. After taking organ weights, these organs were fixed in 10% buffered formalin and submitted for histopathological processing. These organs were processed for histopathology through standard protocols. They

were trimmed, embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin and observed under the microscope at x10, x100 and x400 objective magnification.

3.5 Determination of antihypertensive efficacy of aqueous extracts of *Carpobrotus edulis*

Sprague Dawley rats were housed under conditions described in section 3.4.2. These rats were assigned randomly into five groups of six animals per group as per Table 3.1. The Sprague Dawley rats in all groups were fed with 20% (w/v) fructose solution for 21 days except group E. Group E did not receive any treatments for the entire study period. The 20% fructose solution fed to Sprague Dawley rats was used to induce hypertension. Blood pressure measurements were taken twice weekly (Tuesday and Friday starting 9 am) in order to confirm induction of hypertension. A blood pressure measurement above 120/80 mmHg was considered hypertensive. A non-invasive tail-cuff system (BIOPAC® System Inc., CA) was used to measure blood pressure (Figure 3.3).

The rats then received the differing doses of *Carpobrotus edulis* aqueous extracts for 14 days along with 20% fructose solution treatments in drinking water as per the Table 3.1. Captopril, a conventional antihypertensive drug, was used as a positive control. Blood pressure was measured twice per week (Tuesday and Fridays starting at 9 am) for the entire study period.

Table 3.1: Doses administered in different treatment groups of Sprague Dawley Rats used in evaluation of antihypertensive efficacy of aqueous extracts of *Carpobrotus edulis.*

Treatment	Group of rats (n=6)	Dose Levels (mg/Kg)
Aqueous extract of *C. edulis*	A	300
	B	1000
Captopril	C	50
Normal saline with 20% fructose in water	D	-
Normal saline without 20% Fructose in water	E	-

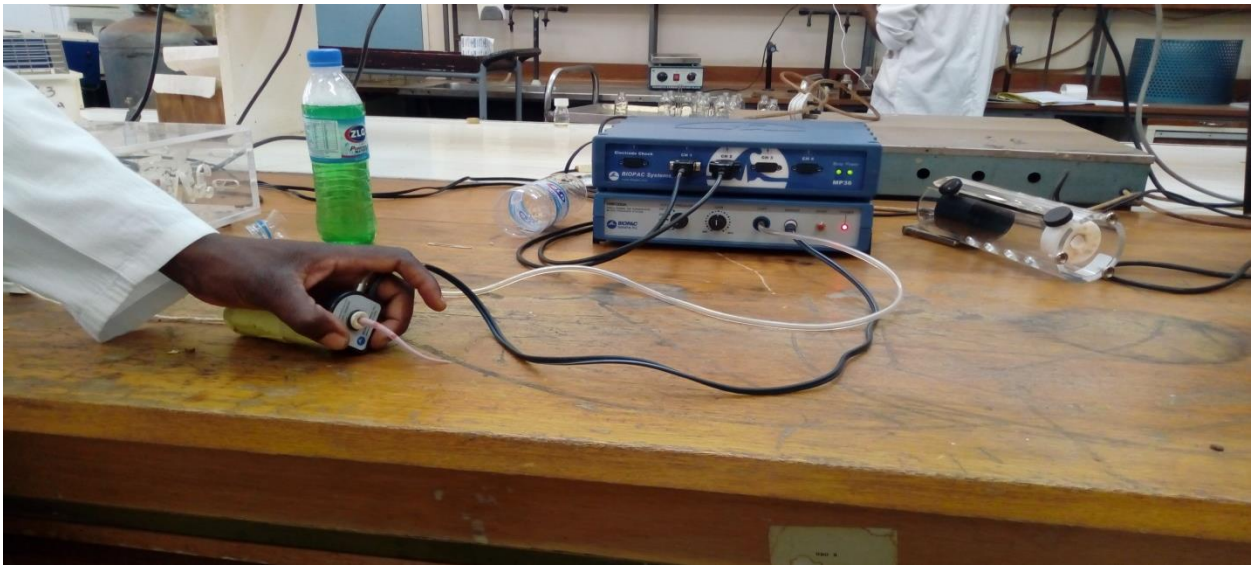

Figure 3.3: Measurement of blood pressure of a Sprague Dawley Rat using the BIOPAC® system

3.6 Statistical analysis

Data was captured on Microsoft Excel and transferred to Statistical Package for the Social Sciences software (SPSS ®version 21.0) for further analysis. Hematological and biochemical parameters were expressed as means ± standard error of the mean (SEM) for all the groups. One-

way ANOVA was used to compare the variation of hematological and biochemical parameters within groups. A 95% level of significance ($p \leq 0.05$) was used in the analysis. Statistical analysis was carried out using one-way ANOVA in order to compare group means of blood pressure measurements and a 95% level of significance ($p \leq 0.05$) was used in the analysis.

CHAPTER FOUR

RESULTS

4.1 Phytochemical composition of aqueous leaf extracts of *Carpobrotus edulis*.

The phytochemical screening results show that eight phytochemical groups were found to be present in the aqueous extracts of *Carpobrotus edulis* as shown in Table 4.1.

Table 4.1: Phytochemical Composition of aqueous extracts of *Carpobrotus edulis* leaves.

Phytochemical group	**Presence/absence in *Carpobrotus edulis* aqueous leaf extract**
Phenols	+
Flavonoids	+
Anthraquinones	+
Alkaloids	+
Terpenoids	+
Saponins	+
Tannins	+
Glycosides	+

+ Denotes presence; - Denotes absence

4.2 Acute oral toxicity effects of aqueous leaf extracts of *Carpobrotus edulis.*

4.2.1 Clinical effects of *Carpobrotus edulis*

There were no mortalities observed during the acute oral toxicity testing of *Carpobrotus edulis* aqueous leaf extract at a dose of 2000 mg/Kg. The animals in all the four treatment groups did not show any notable clinical signs. The weight of rats were taken on weekly basis and the mean weekly weights and the percentage weekly weight increases were tabulated and shown in Table 4.2 and Figure 4.1 respectively. The findings show that aqueous extracts of *Carpobrotus edulis* have no effect on body weights of Sprague Dawley rats.

Table 4.2: Weekly mean weights of Sprague Dawley rat groups used in oral acute toxicity study of *Carpobrotus edulis* aqueous leaf extract

Group (n=3)	Dose levels (mg/Kg)	weight in grams prior *to C. edulis* extract exposure	Weight in grams, one week after exposure	Weight in grams, two weeks after exposure
A	300	121.33 ±10.35	124.00 ±10.06	128.67 ±11.72
B	600	106.33 ±5.90	112.67 ±6.17	127.67 ±8.35
C	1200	131.33 ±19.47	132.00 ±18.77	136.67 ±19.27
D	2000	108.00 ±23.80	116.33 ±20.09	123.33 ±18.48

Values expressed as means± SEM. All the p values of weekly mean weights are greater than 0.05.

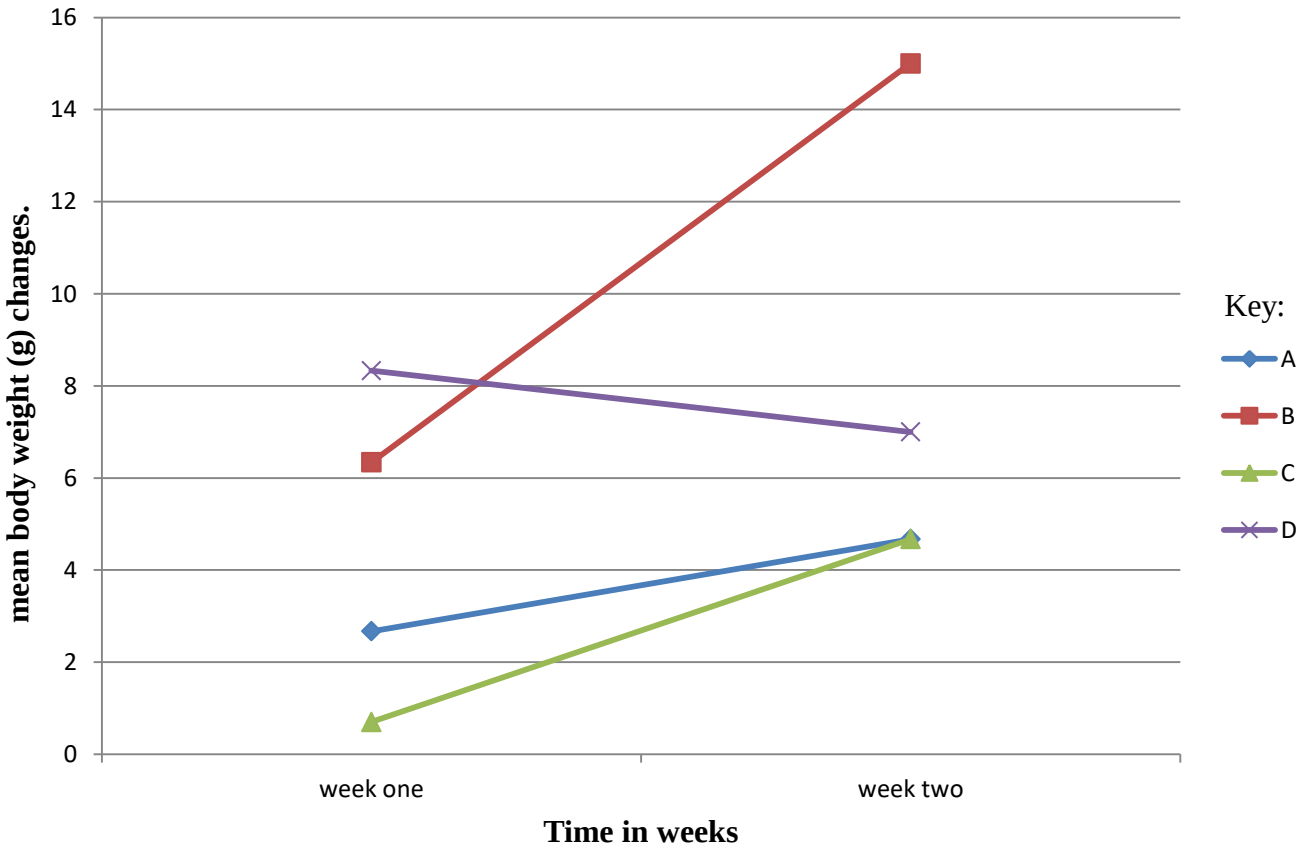

Figure 4.1: Mean body weight changes of rats after oral acute exposure to *Carpobrotus edulis* aqueous leaf extract.

4.2.2 Gross necropsy findings

There were no obvious gross pathological lesions observed in all animals. Histopathology was therefore not done for the animals in acute toxicity because there were no lesions that warranted histopathological processing.

4.3 Subacute toxicity effects of *Carpobrotus edulis* in Sprague Dawley rats

4.3.1 Clinical effects of aqueous extracts of *Carpobrotus edulis* in subacute toxicity testing

There were no unusual behavioural changes noted in the whole study period neither were there any signs suggestive of toxicity in all treatment groups. All the animals in all the four treatment groups survived until scheduled post-mortem examination date. The general body conditions of the animals were not suggestive of any harmful effects of the aqueous extracts of *Carpobrotus edulis* leaves.

Water and feed intake were measured weekly per group. There were no significant changes in water and feed between the treatment groups with p= .08 and p= .25 respectively. Group D (control group) however had consistently higher water and feed intake compared to other treatment groups even though the differences were not statistically significant. Water and feed intake per group are shown respectively in Table 4.3 and Table 4.4.

Table 4.3: Effect of C*arpobrotus edulis* on average daily water intake of Sprague Dawley Rats

Group (n=8)	Dose level (mg/Kg)	Average water intake (ml)			
		Week 1	Week 2	Week 3	Week 4
A	100	95.00±1.94	97.50±4.40	115.14±9.37	98.36±4.24
B	300	96.93±3.31	101.00±5.44	110.43±9.55	107.64±3.78
C	1000	107.79±5.11	107.36±6.59	125.21±10.85	115.64±5.33
D	0	101.79±9.05	131.57±5.24	127.14±9.45	114.64±4.04

Values expressed as mean± SEM. All the p values of average water intake are greater than 0.05.

Table 4.4: Effect of *Carpobrotus. edulis* on Daily feed intake of Sprague Dawley rats

Group (n=8)	Dose level (mg/Kg)	Average feed intake (g)			
		Week 1	Week 2	Week 3	Week 4
A	100	63.86±5.08	70.57±2.50	79.29±3.02	73.79±2.94
B	300	65.00±5.36	70.14±2.74	80.00±3.12	73.93±2.85
C	1000	67.79±5.56	75.43±2.19	80.64±4.26	75.64±3.09
D	-	71.71±6.33	80.71±3.21	86.07±2.76	80.07±3.42

Values expressed as mean± SEM. All the p values of average feed intake are greater than 0.05.

Weight of all the experimental animals were measured on weekly intervals. The mean weights and standard error of the mean (SEM) of the animals in each treatment group are shown in the Table 4.5. Weight increases per group were also calculated. The percentage weight increases per treatment group are shown in Figure 4.2. Group C had an overall high weight increase compared to all the treatment groups while Group B had the lowest. The weight increases differences between groups however were not statistically significant (p=0.608). The findings therefore show that the aqueous extracts of *Carpobrotus edulis* did not have any effects on the body, water and feed intake after 28 days of oral exposure.

Table 4.5: Effects of the *Carpobrotus edulis* aqueous leaf extract on mean body weight (g) of Sprague Dawley rats.

Group (n=8)	Dose level (mg/Kg)	Weekly weights in grams				
		Week 0	Week 1	Week 2	Week 3	Week 4
A	100	160.25 ±20.06	177.63 ±17.31	193.63 ±15.96	209.38 ±15.98	217.63 ±16.53
B	300	168.50 ±16.43	183.75 ±15.34	201.00 ±14.95	214.25 ±14.96	221.88 ±15.40
C	1000	147.38 ±14.14	171.38 ±12.14	198.75 ±13.74	218.50 ±14.56	228.00 ±14.47
D	0	179.25 ±16.77	205.50 ±13.27	227.38 ±13.56	241.88 ±13.56	251.75 ±13.84

Values expressed as mean± SEM. All the p values of body weight are greater than 0.05.

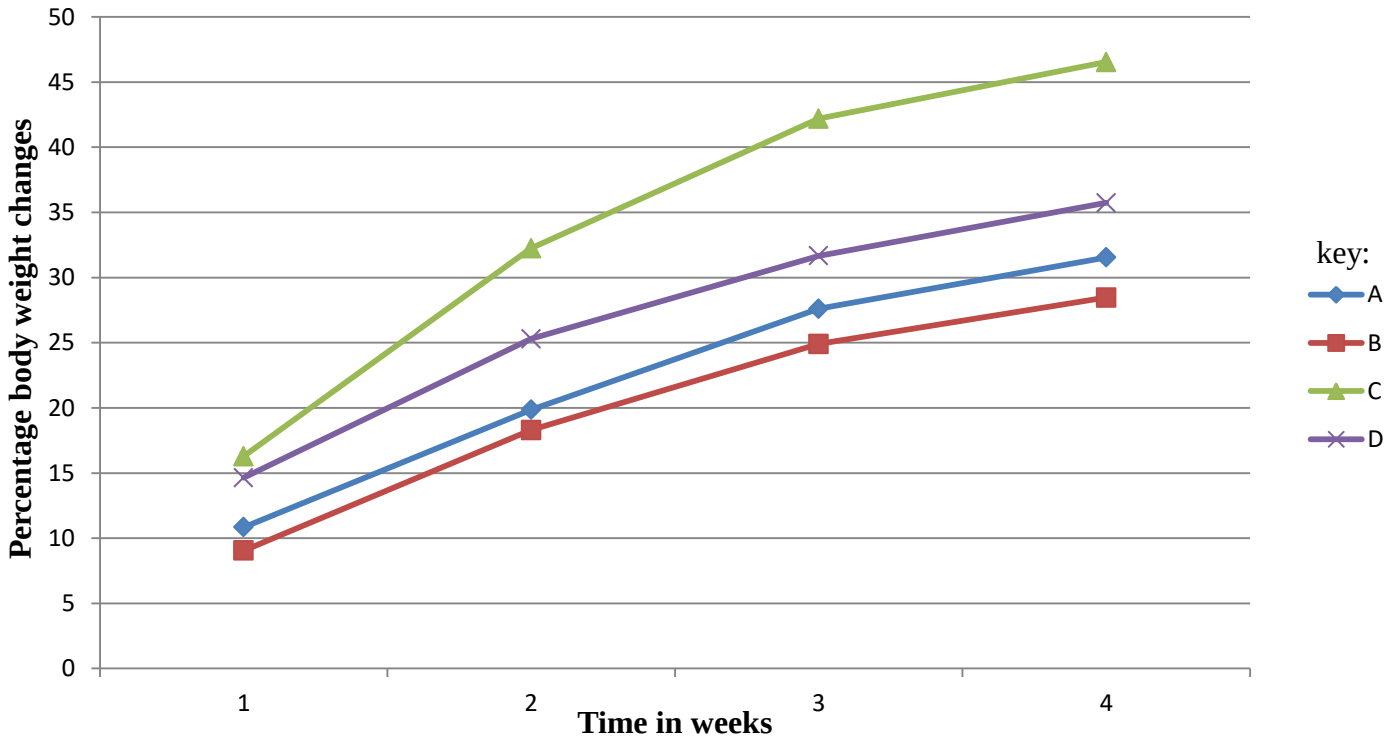

Figure 4.2: Percentage Body weight changes in Sprague Dawley rats treated with *Carpobrotus edulis* leaf aqueous extract in respect to weight at week 0.

4.3.2 Gross Post mortem findings

There were no visible gross pathological lesions seen on all the animals. All the internal organs seemed normal. Organ weights of the heart, liver and kidneys were taken and their mean absolute weights are shown in Table 4.6, while Figure 4.3 shows the percentage mean organ weights relative to mean body weights of animals per group.

Table 4.6: Absolute organ weights of Sprague Dawley rats after 28 day repeated exposure to *Carpobrotus edulis* aqueous leaf extract.

Group (n=8)	Dose (mg/Kg)	Organ weights in grams		
		Liver	Heart	Kidneys
A	100	8.328 ±0.537	1.571 ±0.127	1.703 ±0.148
B	300	8.451 ±0.584	0.982 ±0.081	1.711 ±0.142
C	1000	8.975 ±0.567	1.081 ±0.050	1.942 ±0.824
D	0	9.505 ±0.565	1.118 ±0.057	2.028 ±0.076

Values expressed as mean± SEM. All p values of absolute organ weights are greater than 0.05.

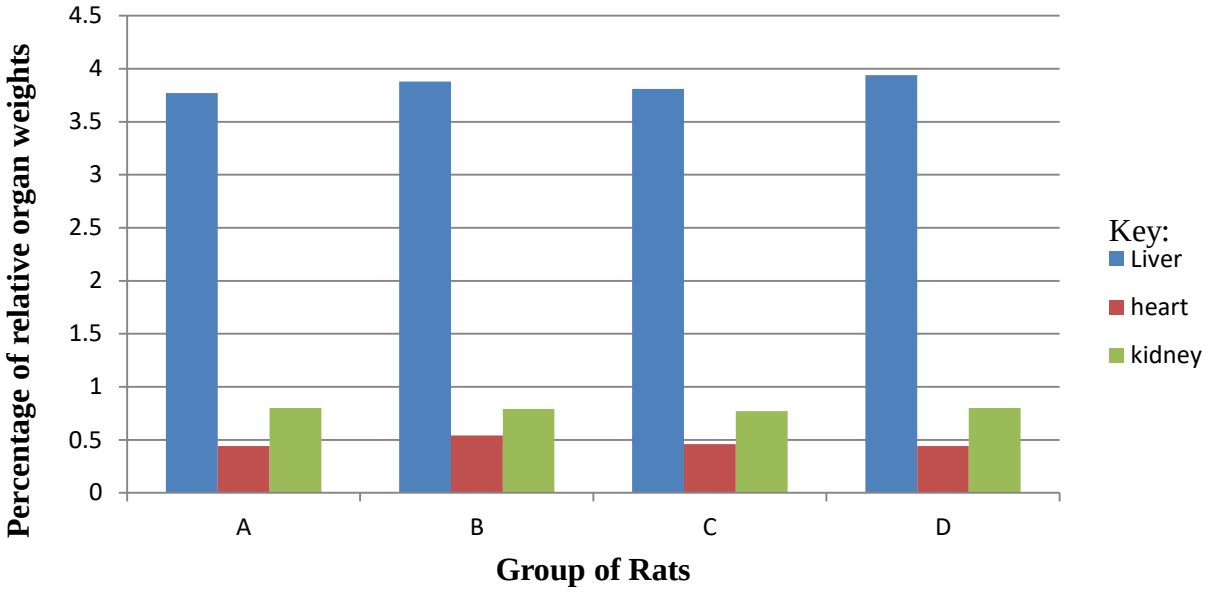

Figure 4.3: Percentage of relative organ weights relative to mean body weights of Sprague Dawley rats after 28-repeated oral exposure to *Carpobrotus edulis* aqueous leaf extract.

4.3.3 Effects of aqueous extracts of *Carpobrotus edulis* on Haematological parameters

There were no significant differences in all measured hematological parameters between the treatment groups of Sprague Dawley rats after 28 day-repeated oral exposure to aqueous

Carpobrotus edulis extracts. Table 4.7 shows the means and SEM of the measured hematological parameters.

Table 4.7: Effects of the *Carpobrotus edulis* aqueous leaf extract on haematological values in Sprague Dawley rats after a 28-day repeated oral exposure.

Hematological indices	Treatment Groups			
	Group A (100 mg/Kg)	**Group B (300 mg/Kg)**	**Group C (100 mg/Kg)**	**Group D (0 mg/Kg)**
WBC ($x10^3$ cells/µL)	6.96 ±0.97	5.93 ±1.22	7.55 ±1.79	7.42 ±1.16
RBC ($x10^6$ cells/µL)	9.21 ±0.30	9.18 ±0.20	9.23 ±0.22	9.45 ±0.21
Hb (g/dL)	17.21 ±0.32	18.06 ±0.38	18.45 ±0.31	18.61 ±0.30
Hct (%)	53.48 ±1.07	52.78 ±1.13	53.45 ±1.09	54.45 ±1.09
MCV (fL)	58.46 ±1.06	57.45 ±0.72	57.87 ±0.62	57.62 ±0.41
MCH (pg/cell)	19.71 ±0.28	19.58 ±0.25	19.71 ±0.40	19.87 ±0.22
MCHC (g/dL)	33.91 ±0.22	34.15 ±0.15	34.52 ±0.29	34.21 ±0.23
RDW (%)	15.09 ±0.57	14.46 ±0.24	13.93 ±0.61	14.60 ±0.30
Platelets ($x10^3$ cells/µL)	534.00 ±30.65	639.25 ±47.93	608.25 ±29.62	576.88±28.75
MPV (fL)	5.35 ±0.78	5.29 ±0.68	5.36 ±0.92	5.48±0.14

Values are expressed as mean ± SEM, n=8. All p values of haematological indices are greater than 0.05.

4.3.4 Effects of aqueous extracts of *Carpobrotus edulis* on biochemical parameters

Various biochemical parameters were evaluated in order to assess the effect of the aqueous *Carpobrotus edulis* extract on hepatic and renal function of Sprague Dawley rats. There were no significant differences on all the measured biochemical parameters between all the treatment groups. The means and SEM of all the measured biochemical parameters are shown in Table 4.8.

Table 4.8: Effect of aqueous extract of *Carpobrotus edulis* leaves on biochemical parameters in Sprague Dawley rats after oral subacute exposure

Biochemical indices	Treatment Groups			
	Group A (100 mg/Kg)	**Group B (500 mg/Kg)**	**Group C (1000 mg/Kg)**	**Group D (0 mg/Kg)**
Total protein (g/L)	59.63 ±1.20	60.15 ±1.50	54.23 ±5.22	51.36 ±4.31
Albumin (g/L)	35.35 ±0.34	41.70 ±6.38	33.26 ±1.60	33.51 ±1.80
ALT (IU/L)	57.93 ±4.81	51.95 ±5.64	58.53 ±2.31	52.44 ±2.86
ALP (IU/L)	136.00 ±7.12	139.28 ±11.56	135.85 ±35.61	132.43 ±20.14
AST (IU/L)	147.65 ±9.48	169.10 ±22.54	179.96 ±71.63	136.40 ±20.10
Urea (mmols/L)	9.50 ±0.43	7.90 ±0.74	9.11 ±1.01	10.11 ±0.78
Creatinine (µmol/L)	180.30 ±8.11	150.33 ±14.18	170.83 ±18.42	190.55±14.22
Total bilirubin (µmol/L)	8.48 ±2.28	12.86 ±4.30	10.79 ±1.65	15.71 ±5.33
Direct bilirubin(µmol/L)	7.23 ±1.93	9.69 ±2.90	9.49 ±1.55	11.90 ±3.94

Values are expressed as mean ± SEM, n=8. All p values of biochemical indices are greater than 0.05.

4.3.5 Histopathological findings

The aqueous extracts of *Carpobrotus edulis* did not show any abnormal effects on the histology of the liver, kidney and the heart. The microscopic pictures of the liver, Kidney and the heart displayed normal histoarchitecture of these organs as shown in Figure 4.4, Figure 4.5, Figure 4.6 and Figure 4.7 respectively.

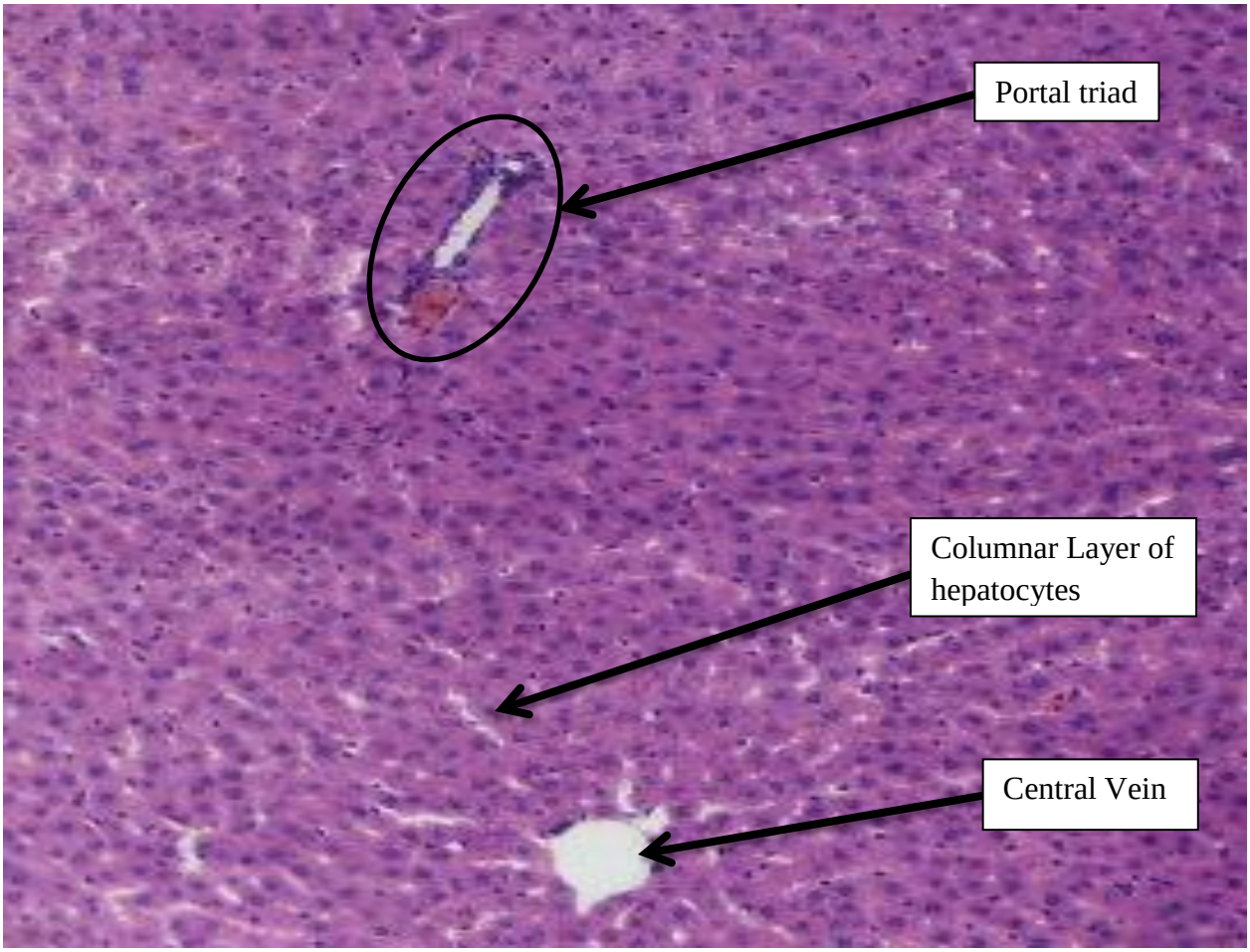

Figure 4.4: The normal liver histoarchitecture of a Sprague Dawley rat that received 1000 mg/Kg dose of aqueous extract of *Carpobrotus edulis* orally for 28 days. (x400)

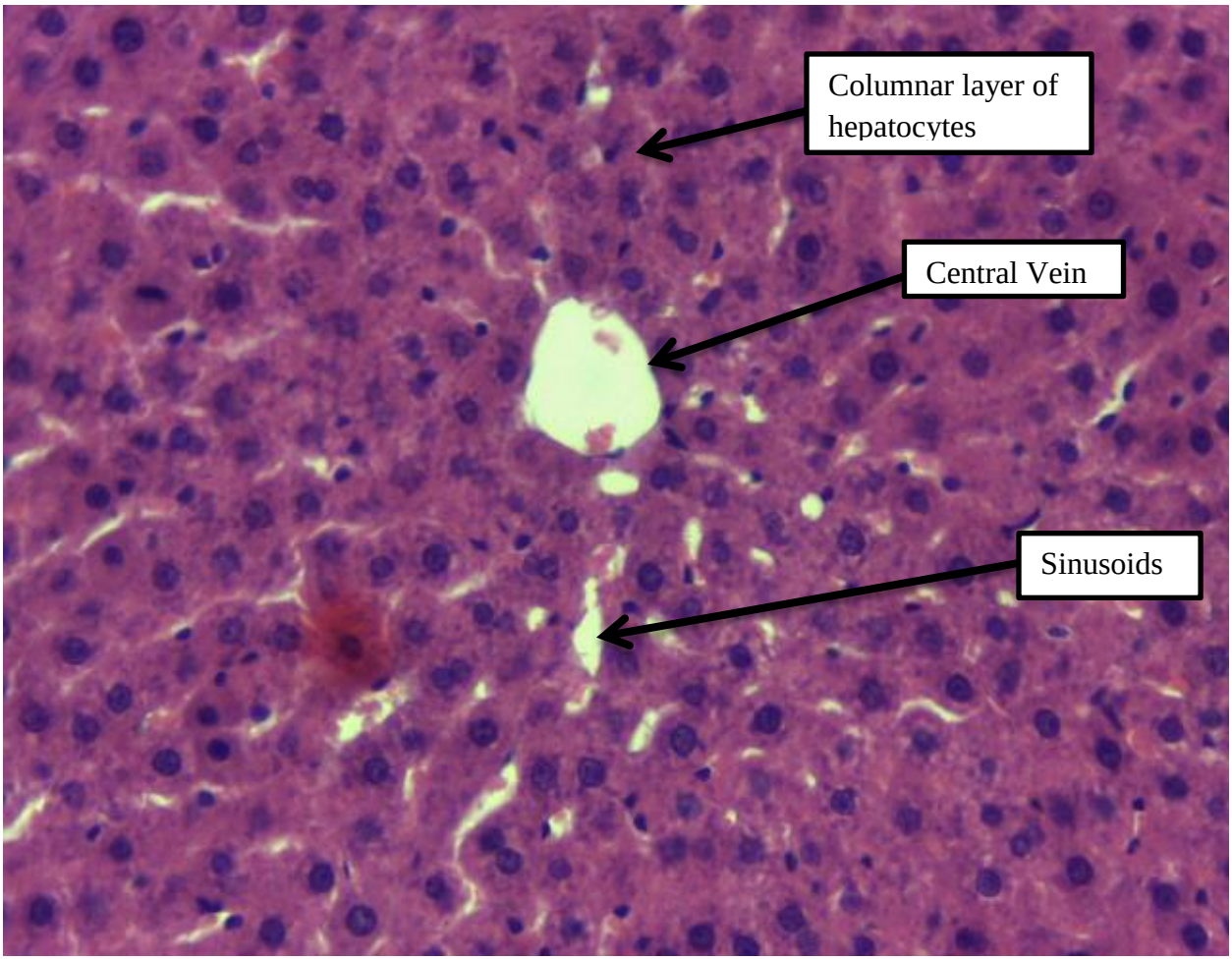

Figure 4.5: The normal Liver histoarchitecture of a Sprague Dawley rat in a negative Control group (x400).

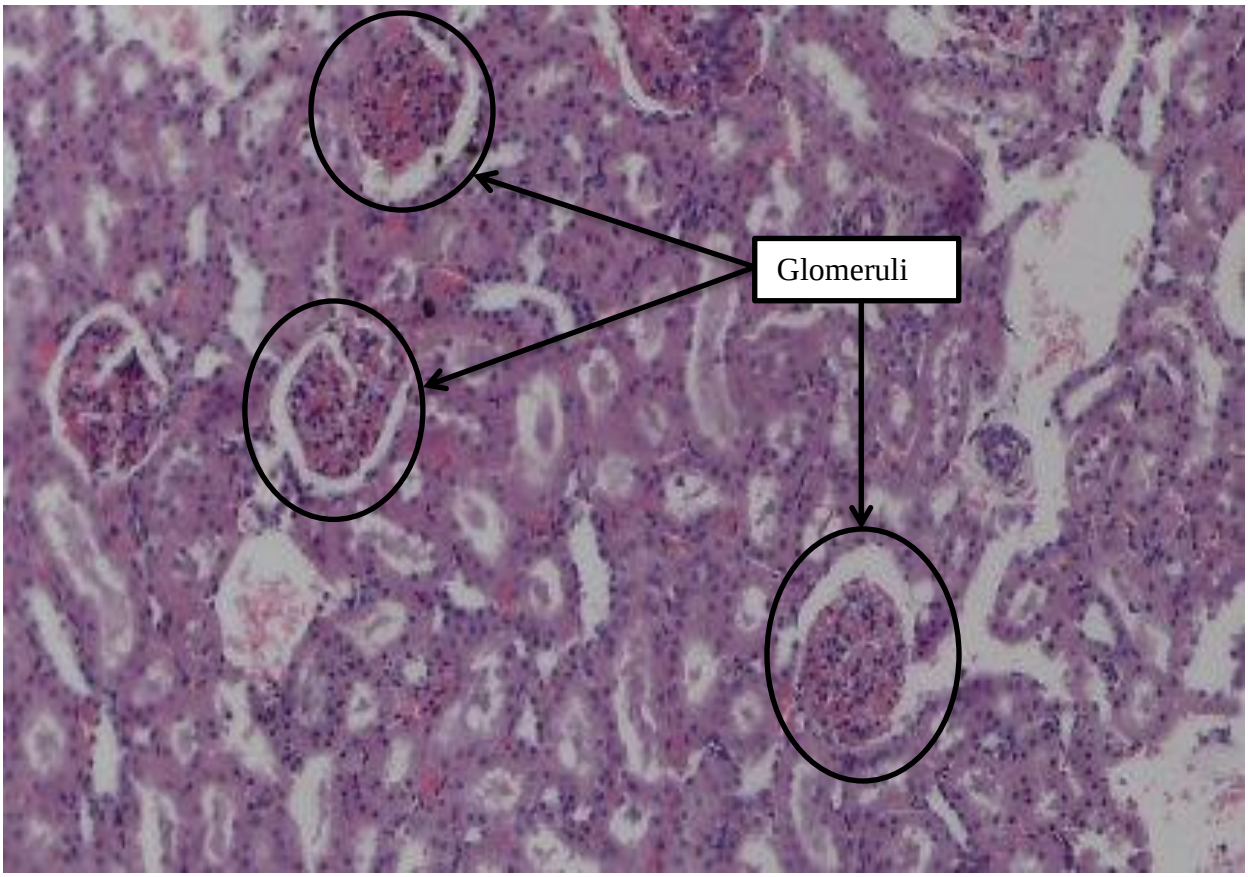

Figure 4.6: The normal histology of the kidney cortex of a Sprague Dawley rat that received 1000 mg/Kg of aqueous extract of *Carpobrotus edulis* orally for 28 days (X400).

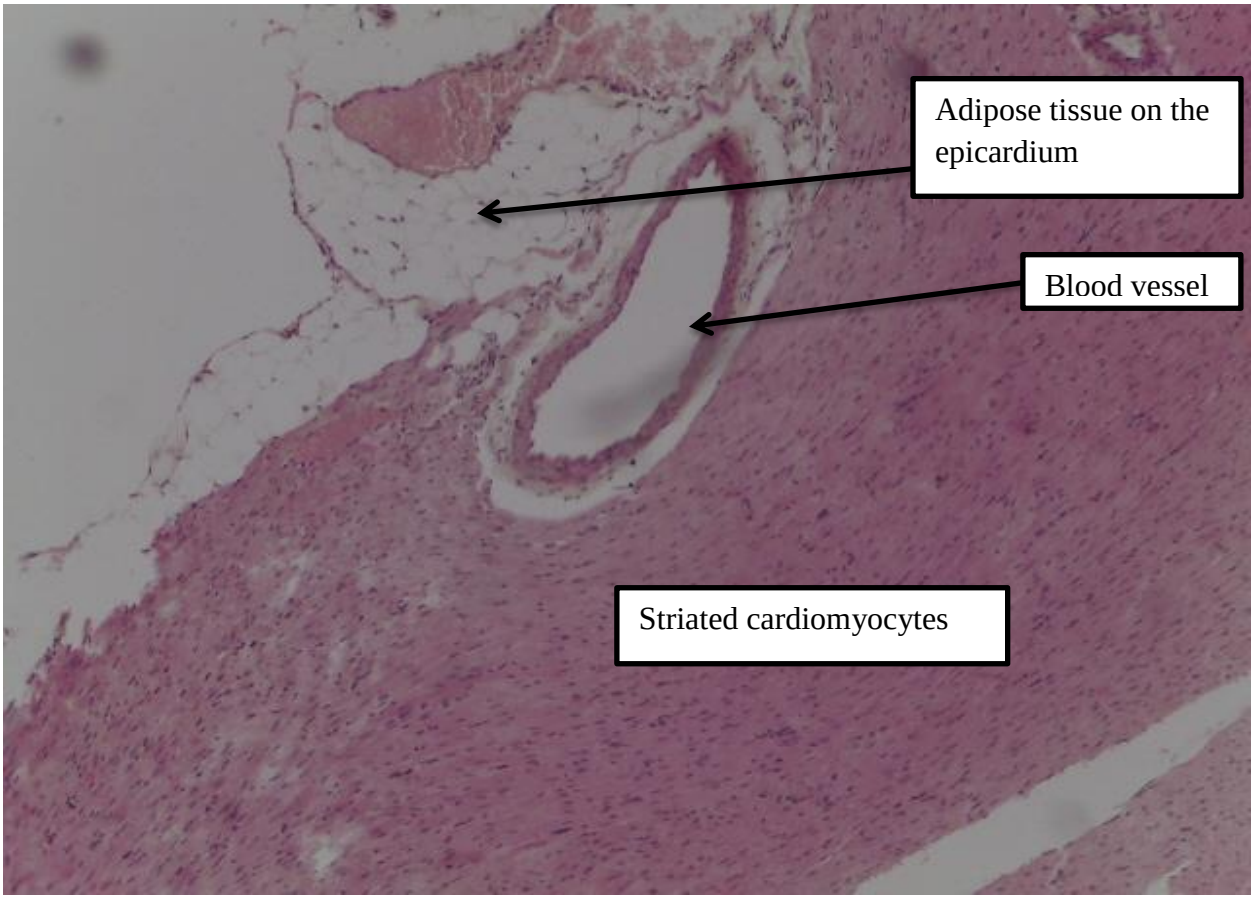

Figure 4.7: The normal histology of the heart of a Sprague Dawley rat that received 1000 mg/Kg of aqueous extract of *Carpobrotus edulis* orally for 28 days (x100)

4.4 Antihypertensive efficacy

4.4.1 Effects of aqueous extracts of *Carpobrotus edulis* on Fructose Induced hypertensive Sprague Dawley rats

Fructose solution (20%) significantly ($P<0.05$) raised both the systolic and diastolic blood pressure of Sprague Dawley rats in Groups A-D. Figure 4.8 and Figure 4.9 shows systolic and diastolic blood pressure changes due to treatment of aqueous extracts of *Carpobrotus edulis*. According to Table 4.9 and Table 4.10, the results show that the aqueous extracts of *Carpobrotus edulis* significantly ($P<0.05$) reduced hypertension at both doses of 300 mg/Kg and 1000 mg/Kg. The aqueous extracts of the plant showed a higher efficacy in reduction of hypertension in Sprague Dawley rats compared to Captopril at 50 mg/Kg.

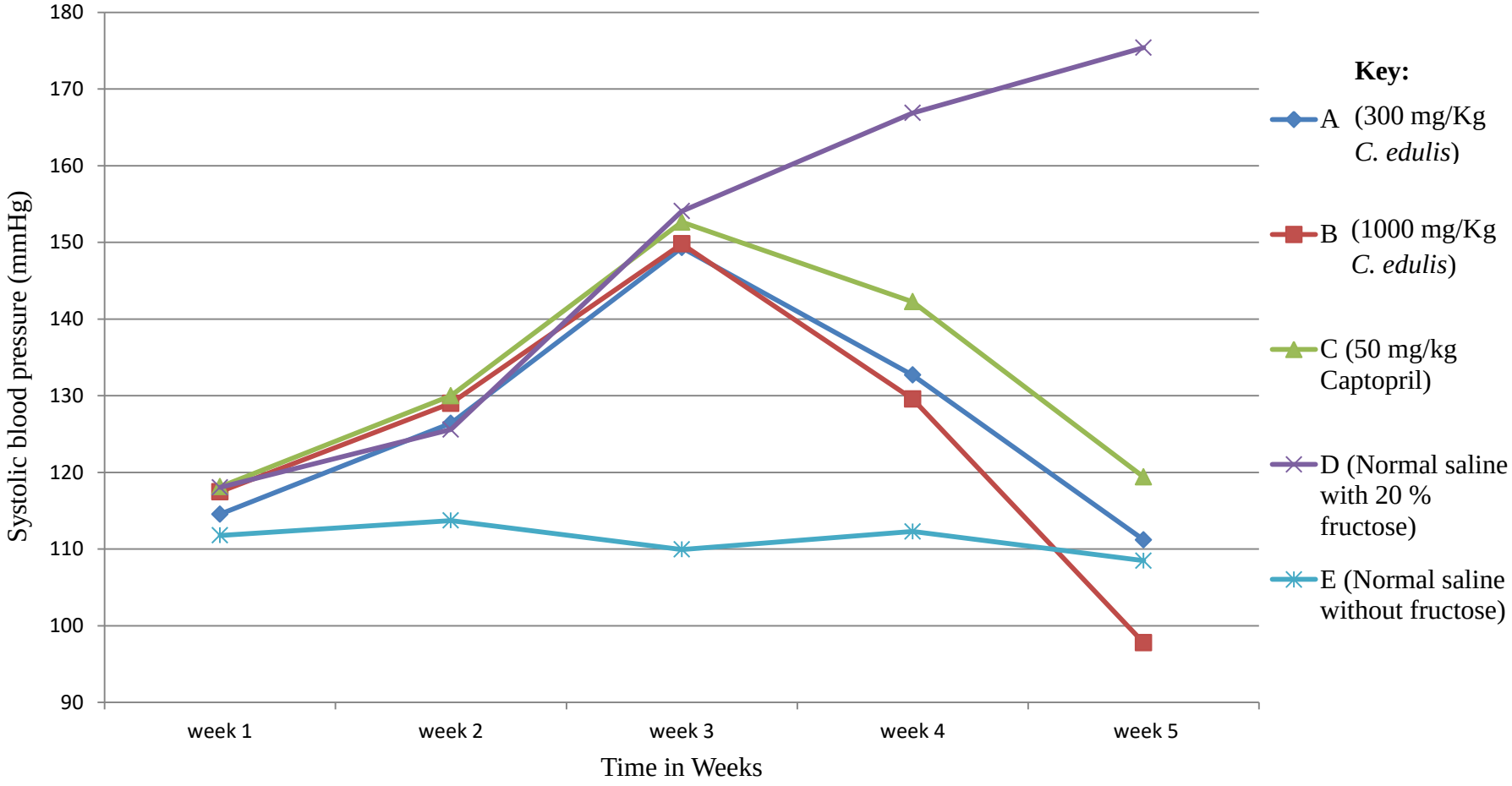

Figure 4.8: Effect of aqueous extracts of *Carpobrotus edulis* on the systolic blood pressure of Fructose-induced hypertensive Sprague Dawley Rats.

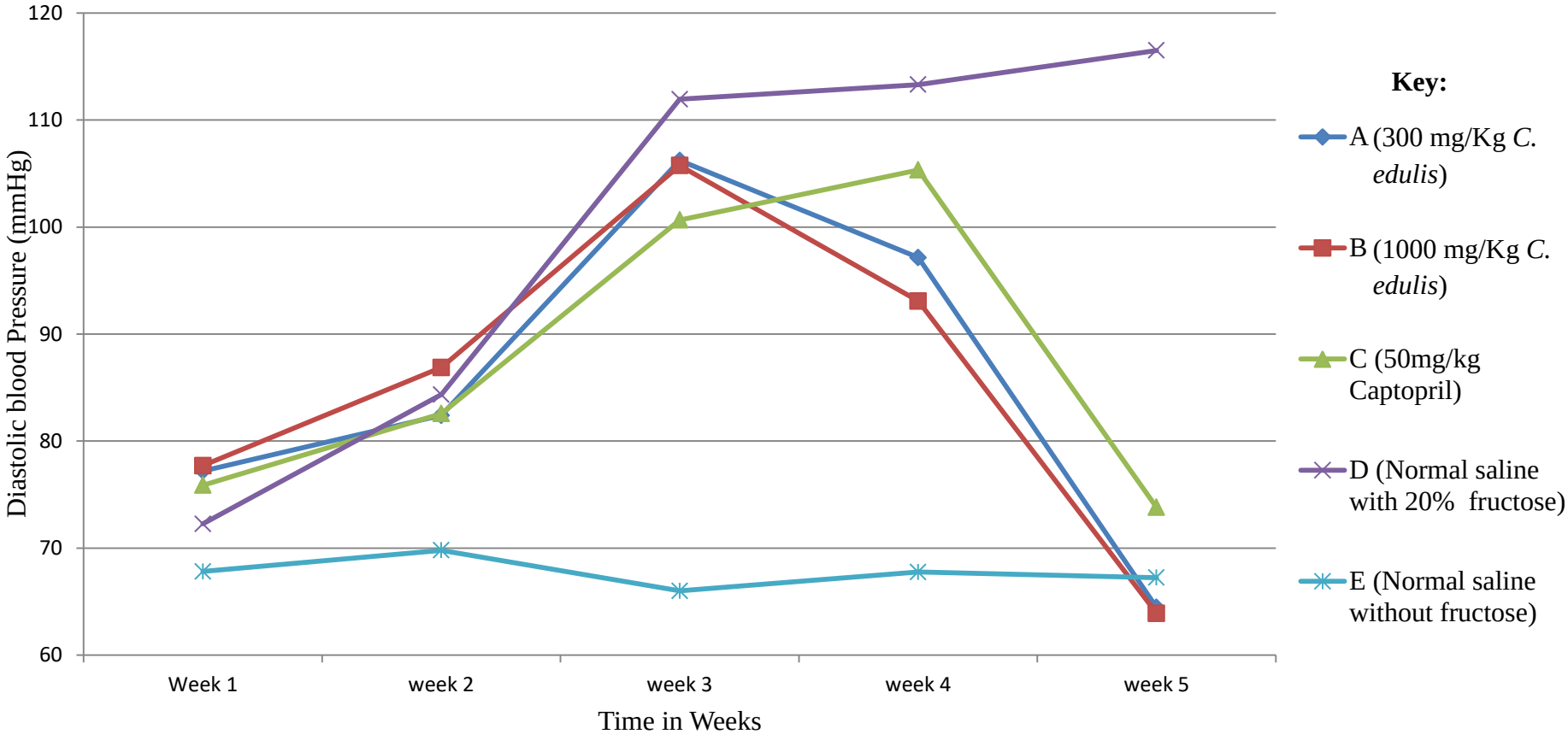

Figure 4.9: Effect of aqueous extracts of *Carpobrotus edulis* on the diastolic blood pressure of Fructose-induced hypertensive Sprague Dawley Rats.

Table 4.9: The effects of *Carpobrotus edulis* on Systolic Blood Pressure of Fructose induced hypertensive Sprague Dawley Rats

Time		Treatment groups				
		A (300 mg/Kg *C. edulis*)	**B (1000 mg/Kg *C. edulis)***	**C (50 mg/Kg Captopril)**	**D (Normal saline with 20% fructose in water)**	**E (Normal saline without 20% Fructose in water)**
Week 1	Tuesday	110.41±2.65	115.44±1.30	116.75±0.33	115.99±0.36	111.93±1.86
	Friday	118.69±0.89	119.51±0.55	119.15±0.36	120.08±0.64	115.60±1.09
Week 2	Tuesday	120.02±0.58*	123.47±0.72*	125.45±0.86*	124.59±0.37*	110.96±1.88
	Friday	132.77±1.06*	134.52±0.58*	127.38±0.56*	126.56±0.32*	116.45±1.34
Week 3	Tuesday	142.81±0.68*	142.76±3.43*	141.12±0.34*	153.56±1.04*	112.09±1.54
	Friday	155.80±1.01*	156.84±1.07*	154.18±0.51*	154.57±0.61*	107.79±2.06
Week 4	Tuesday	140.94±0.81*	140.50±0.86*	133.83±0.90*	165.84±0.99*	108.58±0.47
	Friday	124.43±0.34*	118.65±0.84	130.68±0.53*	167.92±1.08*	115.98±0.43
Week 5	Tuesday	115.31±0.31*	100.7±1.27	123.69±0.76*	172.11±0.55*	104.53±0.88
	Friday	107.07±1.54	94.77±1.57	115.10±0.30	178.68±3.88*	112.41±0.56

Values are expressed as mean ± SEM, n=6. *p value less than 0.05, ($p<0.05$) is statistically significant value against Group E

Table 4.10: The effects of *Carpobrotus edulis* on diastolic blood pressure of Fructose induced hypertensive Sprague Dawley Rats

Time		Experimental Groups				
		A (300 mg/Kg *C. edulis*)	**B (1000 mg/Kg *C. edulis)***	**C (50 mg/Kg Captopril)**	**D (Normal saline with 20% fructose in water)**	**E (Normal saline without 20% Fructose in water)**
Week 1	Tuesday	75.91±1.44	76.84±0.99	75.80±0.22	66.31±0.58	68.92±2.63
	Friday	78.49±0.79	78.55±0.92	75.95±0.28	78.21±1.12	66.69±1.17
Week 2	Tuesday	79.43±0.72	81.74±0.72*	82.90±0.45*	83.01±0.87*	66.46±1.34
	Friday	85.39±0.46*	92.01±0.81*	82.24±0.70*	85.67±0.39*	73.13±2.48
Week 3	Tuesday	99.48±0.63*	99.23±0.90*	89.41±0.33*	112.36±0.91*	64.43±0.62
	Friday	112.93±0.64*	112.27±0.78*	111.91±0.69*	111.51±0.82*	67.48±1.91
Week 4	Tuesday	109.67±0.96*	109.70±0.44*	110.85±0.68*	112.51±0.82*	64.43±0.62
	Friday	84.63±0.98*	76.46±0.64	99.80±0.49*	114.09±0.60*	71.13±0.50
Week 5	Tuesday	67.96±0.60	63.74±0.43	81.64±0.52*	115.49±0.99*	62.25±0.61
	Friday	60.96±1.51	64.03±0.97	65.99±0.27	117.50±0.67*	72.23±0.91

Values are expressed as mean ± SEM, n=6. *p value less than 0.05, ($p<0.05$) is statistically significant value against Group E.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Phytochemicals have been used since time immemorial to treat various types of illnesses. *Carpobrotus edulis* is extensively used in traditional medicine in Southern Africa in order to treat various ailments. The phytochemical screening results of aqueous extracts of *Carpobrotus edulis* indicated presence of crucial secondary plant metabolites such as flavonoids, alkaloids, tannins, phenols, terpenoids, saponins, anthraquinones and glycosides. These results are in accordance with the study done by Eman (2011) who investigated the phytochemical constituents of succulent plants found in Egypt. Phytochemicals usually have a potential of medicinal properties and often serve as lead compounds for the development of new drugs (Uboh *et al*., 2010). The presence of these phytochemicals partly justifies the common herbal use of *Carpobrotus edulis.* The detailed effects of these phytochemicals in normal body physiology are however subject to further investigation since some may have harmful effects in the body. Extracts of *Allium sativa*, for example, increased osmotic fragility of red blood cells in Wistar rats (Salami *et al.*, 2012). Absence of pharmacological and toxicological information on herbal medicines hinders utilization of these products. Man in his quest to find an effective cure of illnesses has been led to aimlessly utilise herbal medicines without focusing on their possible toxicities.

The phytochemicals found in *Carpobrotus edulis* extracts may have harmful effects on biological systems. Safety assessment of the extracts of *Carpobrotus edulis* is therefore valuable in order to reduce a possible toxicological hazard to the exposed population. Acute toxicity study evaluates the toxicological effects of an extract or drug due to a single exposure. A 14 day acute toxicity study of aqueous extract of *Carpobrotus edulis* was performed in Sprague Dawley rats and it

showed no toxicological effects at a dose of 2000 mg/Kg. According to this study, the aqueous extracts of *Carpobrotus edulis* can be classified as non-toxic due to absence of mortalities or any toxic clinical evidence observed at the dose of 2000 mg/Kg (Organisation for Economic Co-operation and Development, 2001).

Body weight is an important indicator of toxicological effects of herbal extracts or drugs (Pariyani *et al.*, 2015). Rapid bodyweight loss of about 15% to 30% within a week provides significant evidence of deleterious physiological effects of the plant extract or drug (Dutok *et al.*, 2015). *Carpobrotus edulis* aqueous extract did not affect the body weight gains in relation to the control group in subacute studies. None of the experimental groups lost weight or gained more weight which could be attributed to the aqueous extract of *Carpobrotus edulis* treatment. The body weight of all the experimental animals followed a normal general trend. Feed and water intake of experimental animals are monitored in toxicological studies because this data gives an insight on the effects of the extracts on the physiology and metabolism of these experimental animals. The differences in feed and water consumption of Sprague Dawley rats in this study in the treatment groups were not statistically significant ($P < 0.05$) compared to the control group.

The evaluation of hematological parameters is very useful in determination of deleterious effects which may be caused by medicinal plants (Uboh *et al*., 2010). Hematological parameters are essential diagnostic tools and such evaluations have higher predictive value on the toxicity of medicinal plants (Mishra and Tandon, 2012). Hematological evaluation carried out for aqueous extracts of *Carpobrotus edulis* on Sprague Dawley rats did not show any significant differences between different treatment groups. White blood cells are responsible for the first response to infectious agents and/ or any tissue injury. Saponins from different medicinal plants are known

to affect white blood cell function negatively (Jimoh *et al.,* 2008). In this study, saponins in the aqueous extracts of *Carpobrotus edulis* did not affect the white blood cell parameters suggesting that the normal physiological mechanisms that maintain white blood cells were not altered.

The insignificant effect of aqueous leaf extract on RBC, MCV, MCH, MCHC , Platelets and MPV show that *Carpobrotus edulis* does not affect the production and/ or the destruction of erythrocytes and thrombocytes. The presence of alkaloids have been associated with the reduction in RBC count (Obeagu, 2018) however, in this study, alkaloids from aqueous extracts of *Carpobrotus edulis* leaves did not have significant effects on red blood cells.

Effects on thrombocyte parameters can have harmful effects since thrombocytes are essential for haemostasis. Some phytochemicals are potential therapeutic agents for thrombocytopenia (Manasa *et al.,* 2016). This was discovered by studying the effects of different extracts on platelets. Phytochemicals in the aqueous extracts of *Carpobrotus edulis* leaves did not affect thrombocytes in Sprague Dawley rats.

Biochemical parameters are key markers in toxicological evaluation as they give information on the deleterious effects of the plant extract to the liver or kidney (Frenzel and Teschke, 2016). The liver and the kidney are the primary organs prone to toxic effects of plant extracts or drugs (Khoo *et al*., 2010). Hepatic function tests and renal function tests are important indicators of toxicity which may not be clinically overt. Plasma urea measurements are key markers of acute kidney function. The rise in plasma urea is usually seen in acute and chronic kidney disease (Park *et al*., 2016). ALT is a specific marker to hepatic damage since only hepatocytes release ALT when damaged (Wurochekke and Usman, 2013). ALT, AST and ALP were measured in order to assess any hepatic damage while plasma urea and plasma creatinine levels were measured in order to evaluate the effects on kidney function. Aqueous extracts of *Carpobrotus*

edulis did not show any statistical difference ($p < .05$) in the hepatic and renal function tests in all experimental groups. All the biochemical parameters measured were within the normal reference ranges in rats. This therefore shows that *Carpobrotus edulis* aqueous extracts do not have any deleterious effects on liver and kidney functions.

Gross pathological changes are important indicators of tissue damages in living organisms. On gross pathological examination, the kidney and liver of Sprague Dawley rats in the treated groups did not show any changes in shape, colour, size and texture when compared to the control group of rats. Relative organ weight is also a significant indicator of physiological status. The liver, kidney, lung and spleen are the primary target organs by toxicants and/or their metabolites (Loha *et al.,* 2019). The liver, kidney and heart relative organ weights were evaluated in this study. Aqueous extracts of *Carpobrotus edulis* did not show any significant effects on relative organ weights in all the experimental groups. The relative organ weight is crucial to assess whether the organs were harmed or not. An astounding change in relative organ weight among treated and untreated experimental animals is a marker of toxicity as organ weight is influenced by the suppression of body weight.

Toxic phytochemicals might result in cellular degeneration and necropsy of body organs. The liver, kidney and heart of Sprague Dawley rats were evaluated for any histopathologic changes. Histology remains the gold standard diagnostic tool for structural related organ damage (McMillan and Harris, 2018). The general histoarchitecture of all the evaluated organs in treated groups did not show any significant differences when compared to the control group. The histopathology results concur with the biochemical results which showed no adverse effects when Sprague Dawley rats were treated with aqueous extracts of *Carpobrotus edulis.*

Treatment with 10% solution of fructose has been known to cause hypertension within three weeks in Wistar rats (Gokhale and Sahu, 2016). In this study, Sprague Dawley rats were given 20% solution of Fructose in drinking water and the hypertension was above 120/80mmHg in two weeks' time period. Fructose is one of the common experimental substances used to induce hypertension. High fructose diet causes hypertension by increasing the activity of sodium and chloride transporters which then leads to a salt overload. It is also reported that excess fructose in the blood inhibits vasodilators while stimulating vasoconstrictors leading to increased peripheral resistance (Soleimani and Alborzi, 2011). Fructose induced hypertension is commonly coupled with hyperinsulinemia and hypertriglyceridemia in Wistar rats. According to Dzeufieta *et al*., (2014), insulin, as a growth factor, promotes vascular smooth muscle proliferation which leads to narrowing of blood vessels thereby causing high peripheral resistance.

The aqueous extracts of *Carpobrotus edulis* showed a dose dependent reduction in blood pressure in Sprague Dawley. These extracts showed the presence of various phytochemicals which can be responsible for antihypertensive effects of *Carpobrotus edulis*. Some Coumarin glycosides have been reported to reduce hypertension through blockage of calcium channels which leads to reduction in cardiac output (Gilani *et al.,* 2000). Flavonoids, like quercetin has also proved to have antihypertensive effects and also being safe to use even in pregnant women (Li *et al*., 2015). The specific phytochemical responsible for antihypertensive effects in the aqueous extracts of *Carpobrotus edulis* remain unknown. Antihypertensive effects of medicinal plants are as a result of different mechanisms which affect the physiological systems associated with the pathophysiology of hypertension. The sympathetic nervous system, excess sodium intake, renin angiotensin system and any disturbances in blood vessels have been implicated in the pathophysiology of hypertension.

5.2 Conclusions

The following conclusions were made from this study.

i. Aqueous leaf extracts of *Carpobrotus edulis* which grow in Zimbabwe contain important phytochemicals. These phytochemicals have potential medicinal properties and that is why it is a common herbal medicinal plant in Southern Africa.

ii. The oral LD_{50} of the *Carpobrotus edulis* aqueous extract was found to be above 2000 mg/Kg and is therefore classified as less toxic.

iii. In the subacute toxicity study, there were no adverse effects observed on bodyweight, feed/water intake of Sprague Dawley rats. It is concluded that the phytochemicals in aqueous leaf extracts of *Carpobrotus edulis* do not have harmful effects on hematological and biochemical parameters of Sprague Dawley rats.

iv. The aqueous extracts of *Carpobrotus edulis* reduced blood pressure significantly in Fructose induced hypertensive rats. The study therefore successfully validated experimentally the antihypertensive efficacy of *Carpobrotus edulis* in fructose-induced hypertensive rats, as it is commonly used in herbal medicine.

5.3 Recommendations

The following recommendations were made from the studies.

i. Further chronic toxicity studies on different *Carpobrotus edulis* extracts should be done in order to ascertain the long term safety profile of the medicinal plant.

ii. It is therefore recommended that further research should be done on the antihypertensive mechanism of action of *Carpobrotus edulis* extracts.

iii. It is also recommended to investigate on the possible drug-herb interaction of *Carpobrotus edulis* extracts with common antihypertensive drugs.

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APPENDICES

Appendix 1: Raw haematology Results of Sprague Dawley Rats in Subacute toxicity.

FACULTY OF VETERINARY SCIENCE
CLINICAL STUDIES DEPARTMENT

Clinical pathology section

DR MUDIMBA HAEMATOLOGY RESEARCH WORKSHEET

GRP	SEX	Animal #	WBC	RBC	Hb	Hct	MCV	MCH	MCHC	PLT	RDW	MPV
1	M	1	9.20	10.20	20.10	60.30	59.30	19.70	33.20	515.00	15.20	5.40
1	M	2	3.10	8.90	17.10	51.90	58.00	19.30	33.30	587.00	15.20	5.30
1	M	3	7.50	10.30	19.00	53.50	53.50	18.50	34.60	506.00	16.00	5.20
1	M	4	7.70	10.00	18.80	54.60	54.60	18.70	34.30	409.00	15.70	5.20
2	M	5	10.10	10.00	20.40	59.70	59.50	20.30	34.10	615.00	15.00	5.60
2	M	6	4.20	9.40	18.00	52.40	55.60	19.00	34.30	473.00	14.50	5.10
2	M	7	12.20	9.10	17.70	51.70	57.00	19.40	34.20	812.00	14.90	5.20
2	M	8	4.00	9.90	18.20	53.30	53.80	18.30	34.10	552.00	15.40	5.50
3	M	9	18.50	9.20	18.70	53.70	58.50	20.30	34.80	518.00	14.90	5.70
3	M	10	6.60	9.50	18.90	54.20	56.90	19.80	34.80	558.00	14.30	5.70
3	M	11	3.70	10.10	19.80	55.70	55.10	19.50	35.50	549.00	14.50	5.50
3	M	12	9.30	10.10	18.90	57.20	56.60	16.60	33.00	601.00	15.50	5.40
4	M	13	11.60	9.70	18.80	54.80	56.60	19.40	34.30	598.00	14.60	5.80
4	M	14	8.70	10.40	20.00	60.00	57.70	19.40	33.80	578.00	15.20	5.00
4	M	15	9.70	9.40	18.20	53.60	57.10	19.30	33.90	622.00	14.90	5.80
4	M	16	5.00	9.90	19.20	57.70	58.30	21.00	33.20	563.00	15.80	5.00
1	F	17	10.00	8.40	17.70	51.30	61.00	20.40	34.50	641.00	14.20	5.50
1	F	18	2.90	8.40	17.20	50.80	60.50	20.40	33.80	622.00	13.60	5.10
1	F	19	9.30	9.10	18.40	53.40	58.90	20.20	34.40	422.00	18.00	5.80

1	F	20	6.00	8.40	17.30	52.10	61.90	20.50	33.20	570.00	12.80	5.30
2	F	21	3.60	8.90	17.40	51.80	58.20	19.50	33.40	481.00	13.50	5.50
2	F	22	2.30	8.40	16.60	48.60	57.80	19.70	34.10	820.00	13.70	5.10
2	F	23	4.70	8.90	17.90	51.00	57.50	20.10	35.00	711.00	14.00	5.30
2	F	24	6.40	8.90	18.30	53.80	60.20	20.40	34.00	650.00	14.70	5.00
3	F	25	4.70	8.60	17.60	49.70	57.70	20.40	35.40	574.00	10.00	5.20
3	F	26	9.80	9.10	18.70	54.70	60.50	20.60	34.10	693.00	15.00	5.00
3	F	27	3.80	8.50	17.40	50.90	59.80	20.40	34.10	771.00	13.50	5.30
3	F	28	4.00	8.80	17.60	50.90	57.90	20.00	34.50	602.00	13.70	5.10
4	F	29	11.10	9.80	19.10	54.50	55.70	19.50	35.00	411.00	14.90	5.10
4	F	30	5.50	8.90	18.10	51.50	58.10	20.40	35.10	589.00	13.00	5.90
4	F	31	5.00	8.80	17.40	51.20	58.50	19.80	33.90	701.00	14.60	5.50
4	F	32	2.80	8.90	18.00	52.30	59.00	20.20	34.40	553.00	13.80	5.70

Key:

Haematology parameters' measurement units are as follows;

WBC ($x10^3$ cells/µL), RBC ($x10^6$ cells/µL), Hb (g/dL), Hct (%), MCV (fL), MCH (pg/cell), MCHC (g/dL), RDW (%), Platelets ($x10^3$ cells/µL), MPV (fL)

Appendix 2: Raw Biochemistry results of Sprague Dawley Rats in Subacute toxicity.

FACULTY OF VETERINARY SCIENCE
CLINICAL STUDIES DEPARTMENT

Clinical pathology section

DR MUDIMBA BIOCHEMISTRY RESEARCH WORKSHEET

Animal #	SEX	GRP	ALP	Creat	Alb	ALT	TP	AST	Urea	Tbil	Dbil
1	M	1	156.40	174.80	36.20	57.60	62.10	160.90	9.20	13.50	12.00
2	M	1	127.30	210.90	33.60	48.60	54.80	159.10	11.10	5.00	4.20
3	M	1	141.80	169.10	35.30	58.60	57.70	177.70	8.90	4.20	3.60
4	M	1	171.40	165.20	35.40	44.20	60.50	163.80	8.80	10.90	8.30
5	M	2	171.50	191.80	37.20	30.20	67.00	179.90	10.00	35.80	20.60
6	M	2	146.00	178.60	33.10	84.20	55.40	145.50	9.40	5.80	4.60
7	M	2	148.90	207.10	86.30	47.60	65.70	311.00	10.90	28.40	24.50
8	M	2	191.40	167.20	35.40	49.50	58.40	135.70	8.80	7.40	6.10
9	M	3	132.70	193.80	34.40	70.10	59.80	133.30	10.20	10.00	9.00
10	M	3	370.00	186.50	34.90	55.20	58.10	675.80	10.00	19.40	17.20
11	M	3	57.00	230.60	33.10	65.00	58.70	87.60	12.40	11.00	9.60
12	M	3	142.20	214.70	37.20	57.30	62.10	119.50	11.30	9.60	8.50
13	M	4	145.00	209.00	33.60	56.20	55.30	115.90	11.00	5.90	3.40
14	M	4	146.50	165.30	35.10	47.90	57.20	133.10	8.70	4.40	3.60
15	M	4	156.10	235.60	34.60	45.20	59.30	256.40	12.40	17.20	15.10
16	M	4	136.70	178.60	35.70	58.30	59.50	126.50	9.40	45.40	30.60
17	F	1	130.90	152.40	36.70	60.10	65.30	111.20	8.00	4.40	3.90
18	F	1	124.80	197.60	35.90	49.80	61.80	149.50	10.40	21.60	18.40
19	F	1	128.70	161.50	34.90	55.80	57.90	157.50	8.50	5.10	4.60
20	F	1	106.70	210.90	34.80	88.70	56.90	101.50	11.10	3.10	2.80

21	F	2	100.70	117.80	35.70	41.20	58.20	179.80	6.20	8.00	7.40
22	F	2	104.40	106.40	34.90	59.60	60.90	96.50	5.60	3.40	2.00
23	F	2	109.70	114.00	36.10	45.80	59.70	169.10	6.00	8.80	7.60
24	F	2	141.60	119.70	34.90	57.50	55.90	135.30	6.30	5.30	4.70
25	F	3	48.50	87.40	23.50	56.50	26.10	55.00	4.60	6.80	5.60
26	F	3	100.40	121.60	30.20	52.80	36.50	91.40	6.40	5.30	4.20
27	F	3	103.80	125.40	36.40	61.00	65.20	130.20	6.60	15.80	14.50
28	F	3	132.20	206.60	36.40	50.30	67.30	146.90	11.40	8.40	7.30
29	F	4	105.20	151.30	35.80	53.80	47.50	121.60	8.00	3.10	2.50
30	F	4	58.20	129.20	22.00	66.00	26.20	55.50	6.80	14.20	8.50
31	F	4	240.80	235.10	39.20	40.20	63.10	179.50	12.90	5.20	4.80
32	F	4	70.90	220.30	32.10	51.90	42.80	102.70	11.70	30.30	26.70

KEY:

Biochemistry parameters' measurement units are as follows:

Total protein (g/L), Albumin (g/L), ALT (IU/L), ALP (IU/L), AST (IU/L), Urea (mmols/L), Creatinine (µmol/L), Total bilirubin (µmol/L), Direct bilirubin(µmol/L)

Appendix 3: Systolic blood pressure (mmHg) measurements of Sprague Dawley Rats

Animal id	Group	Week 1[a]	Week 1[b]	Week 2[a]	Week 2[b]	Week 3[a]	Week 3[b]	Week 4[a]	Week 4[b]	Week 5[a]	Week 5[b]
1	1	118.24	120.60	120.07	135.26	142.67	159.45	140.07	125.36	115.29	109.25
2	1	109.42	117.67	122.24	134.43	145.77	156.20	144.77	124.25	114.6	108.21
3	1	106.50	121.04	120.44	130.22	143.25	157.21	139.25	125.36	114.27	105.22
4	1	100.27	118.11	118.45	132.36	142.22	152.22	140.21	124.21	115.26	100.21
5	1	112.58	115.12	120.45	135.11	141.22	154.50	141.25	124.22	116.42	110.25
6	1	115.42	119.57	118.46	129.21	138.27	155.22	140.10	123.20	116.25	109.25
7	2	118.21	120.24	125.52	136.39	139.42	158.20	140.22	119.21	100.20	100.08
8	2	115.52	117.45	124.56	135.26	138.18	156.37	139.53	115.42	96.57	95.36
9	2	113.25	119.61	123.55	132.55	137.24	152.15	138.15	118.66	101.29	91.06
10	2	118.22	118.41	124.21	134.14	140.62	156.42	140.40	117.69	98.61	93.16
11	2	117.20	120.33	120.52	135.42	142.64	158.62	144.45	121.62	105.55	97.46
12	2	110.22	121.01	122.44	133.35	138.42	159.49	141.27	119.31	102.42	90.31
13	3	117.48	120.67	127.15	128.45	141.52	155.50	155.13	130.63	125.17	115.28
14	3	115.47	119.28	126.17	128.46	140.27	153.28	156.47	129.32	124.15	116.26
15	3	117.08	119.31	125.18	127.11	141.58	154.65	154.30	129.14	124.38	115.16
16	3	116.30	118.43	126.31	126.1	141.19	152.14	150.15	132.61	125.36	116.24
17	3	117.30	118.12	126.38	128.61	141.63	154.43	152.62	130.25	120.46	115.32
18	3	116.59	119.14	121.39	125.24	140.53	155.09	154.37	120.66	122.61	117.02
19	4	116.52	120.70	125.49	126.52	155.23	155.26	170.41	170.32	172.45	175.43
20	4	115.49	121.17	124.67	127.32	150.44	152.27	165.35	164.19	171.15	179.53
21	4	115.0	119.55	125.46	125.40	156.25	155.61	166.39	169.45	173.16	180.54
22	4	116.57	117.32	123.12	126.17	153.48	154.25	163.47	170.22	174.04	180.45
23	4	115.16	120.04	124.70	126.38	150.52	153.61	164.33	168.19	170.44	179.61
24	4	117.21	121.72	124.07	127.56	155.46	156.39	165.11	165.13	171.46	176.52
25	5	117.30	110.39	112.52	118.23	115.22	109.66	108.13	115.42	105.39	112.21
26	5	110.65	112.31	114.24	118.42	105.41	100.32	109.54	117.44	107.55	113.25
27	5	105.18	113.41	109.63	112.63	112.46	112.20	107.52	116.39	103.23	112.42
28	5	116.62	115.41	102.25	116.34	15.61	107.55	110.37	115.25	105.35	110.45
29	5	112.22	109.55	112.65	120.57	110.54	103.58	108.38	116.74	104.46	114.51
30	5	109.63	108.20	114.44	112.50	113.31	113.39	107.51	114.64	101.19	111.61

Appendix 4: Diastolic blood pressure (mmHg) measurements of Sprague Dawley Rats

Animal id	Group	Week 1[a]	Week 1[b]	Week 2[a]	Week 2[b]	Week 3[a]	Week 3[b]	Week 4[a]	Week 4[b]	Week 5[a]	Week 5[b]
1	1	78.01	78.67	79.67	86.07	100.45	115.00	110.55	86.22	67.25	60.23
2	1	75.89	75.23	78.66	85.21	97.21	113.20	112.25	85.24	68.25	63.45
3	1	79.01	78.23	77.34	83.22	99.25	113.45	110.20	86.27	65.37	64.21
4	1	69.59	81.21	79.01	85.76	100.52	113.22	110.23	83.51	69.45	63.21
5	1	74.49	78.53	82.66	86.42	101.22	112.44	105.25	86.29	68.42	60.42
6	1	78.49	79.11	79.24	85.66	98.23	110.25	109.46	80.26	69.21	64.21
7	2	79.01	81.29	84.25	95.21	100.27	112.14	110.16	76.47	63.34	60.08
8	2	73.53	80.55	81.42	91.22	101.28	110.52	109.39	74.10	65.19	64.07
9	2	76.61	77.45	79.45	90.23	95.14	13.06	108.14	78.08	64.38	63.15
10	2	78.49	75.44	83.34	91.57	98.66	114.65	109.13	78.24	62.14	65.26
11	2	74.36	79.49	81.44	93.55	100.54	113.64	111.21	76.45	63.25	67.17
12	2	78.64	77.09	80.59	90.27	99.49	112.63	110.22	75.43	64.12	64.35
13	3	75.49	76.27	82.47	80.64	89.15	112.38	112.68	100.53	82.69	66.11
14	3	76.49	75.11	81.17	84.12	89.50	114.02	108.16	99.52	80.41	67.09
15	3	75.70	76.61	82.63	84.42	89.41	113.11	111.33	100.35	81.14	65.41
16	3	75.52	76.77	83.36	80.35	89.44	110.41	112.40	97.71	83.65	66.05
17	3	76.40	75.55	83.38	82.31	90.73	109.45	110.31	99.50	80.62	66.03
18	3	75.17	75.39	84.40	81.57	88.21	112.09	110.19	101.21	81.33	65.21
19	4	67.17	75.05	83.26	85.62	112.55	112.17	115.18	115.39	112.06	119.14
20	4	66.40	76.01	82.13	86.20	113.16	112.59	114.31	115.48	113.55	118.41
21	4	66.28	80.15	84.49	85.60	115.42	113.15	112.33	115.20	117.28	115.05
22	4	64.18	80.41	86.23	87.02	110.34	111.44	110.40	113.62	118.64	117.17
23	4	65.51	81.42	80.31	85.42	109.24	109.40	110.22	112.21	116.18	116.24
24	4	68.31	76.21	81.64	84.14	113.45	110.23	112.61	112.66	115.26	119.03
25	5	76.52	65.17	69.42	79.62	75.36	64.47	65.13	70.24	61.22	73.38
26	5	61.42	67.26	67.54	69.35	74.48	70.21	62.54	72.61	63.44	69.37
27	5	63.25	69.35	64.61	68.56	73.64	68.60	63.42	72.41	60.34	72.37
28	5	75.36	70.58	61.43	72.45	71.40	65.38	66.51	70.34	62.58	70.20
29	5	71.59	63.38	65.51	81.59	60.19	61.53	65.56	71.54	64.36	75.54
30	5	65.37	64.40	70.26	67.18	62.49	74.67	63.44	69.64	61.53	72.52

Appendix 5: The freeze dryer (Edwards-Freeze Dryer Modulyo, EF4) used to freeze dry the *Carpobrotus edulis* aqueous extract.

Appendix 6: A technician explaining about the BIOPAC® System to the Lead Supervisor Prof J. Mbaria.

Appendix 7: Proposal Approval by the Graduate School

UNIVERSITY OF NAIROBI
GRADUATE SCHOOL

Telephone: 3318262
Fax Number: 243626
Telegrams:"Varsity of Nairobi"
E-mail: gs@uonbi.ac.ke
P. O. Box 30197 - 00100
NAIROBI, KENYA

Our Ref: J56/7285/2017

6th June, 2019

Dr. Mudimba Toonse Nguwesu
C/o Dept. of PHPT
FACULTY OF VETERINARY MEDICINE, CAVS

Dear Dr. Mudimba,

RESEARCH PROPOSAL AND SUPERVISORS

This is to inform you that the Director, Graduate School has approved your MSc. research proposal titled **"Study of Phytochemistry, Toxicity and Antihypertensive Activity of carpobrotus *Edulis Aqueous* Leaf Extract."**

She has also approved **Prof. James M. Mbaria, Prof. Timothy Maitho** and **Dr. Tafadzwa Taderera** as the supervisors of your thesis.

You should therefore begin consulting them and ensure that you submit your thesis for examination in **November 2019**. The Guidelines on Postgraduate Supervision can be accessed on our website (www.gs.uonbi.ac.ke) while the Research Notebook is available at the University Bookstore.

Yours sincerely,

B. MWANGI (MR)
FOR: DIRECTOR, GRADUATE SCHOOL

cc Dean – Faculty of Veterinary Medicine
Chairman – Department of PHPT
Prof. James M. Mbaria – C/o Department of PHPT
Prof. Timothy Maitho – C/o Department of PHPT
Dr. Tafadzwa Taderera – C/o University of Zimbabwe, Department of Physiology

BM/lwk

Appendix 8: Biosafety, Animal Use and Ethics Committee Approval from the Faculty of Veterinary Medicine, University of Nairobi

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

REF: FVM BAUEC/2019/226

Dr. Mudimba Toonse Nguwesu
University of Nairobi
Dept. of PHP & Toxicology

12/06/2019

Dear Dr. Nguwesu,

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Study of Phytochemistry, Toxicity and Antihypetensive activity of *Carpobrotus edulis* aqueous leaf extract.

By Dr. Mudimba Toonse Nguwesu Reg Number J56/ 7285/ 2017

We refer to your MS.c proposal submitted to our committee for review and your application letter dated 21st May 2019.

We have reviewed your application for ethical clearance while undertaking the *in vivo* toxicity and antihypertensive studies using wistar rats and tissue processing thereafter and are satisfied that the procedure meets acceptable minimum standards of the Faculty ethical regulation guidelines. The proposed number of animals meets the 3R principle guidelines.

We have also noted that registered veterinary surgeons will supervise the work.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee
Faculty of Veterinary Medicine.

Appendix 9: Ethical approval granted by the Animal Research Ethics and Animal Welfare Sub-committee in the Department of Livestock and Veterinary Services of Zimbabwe.

All communications should be addressed to "Director Division of Veterinary Services"

Telephone: 263-4-706604/ 263-4-705885-7
Fax: 263-4-791516
Email: nyika56@gmail.com
Website: www.vetservices.org.zw

ZIMBABWE

Reference:

DIVISION OF VETERINARY SERVICES
DEPARTMENT OF LIVESTOCK AND VETERINARY SERVICES
Ministry of Agriculture, Mechanisation and Irrigation Development
P O Box CY 56, Causeway
Harare

01 March 2019

TO: Dr Toonse Mudimba
College of Agriculture and Veterinary Sciences
P.O Box 29053
Loresho Ridge
Nairobi

Dear Dr Mudimba

RE: Use of Experimental Animals for Research Projects Approval (Ref Number 001/2019)

We have the pleasure to inform you that the Animal Research Ethics and Animal Welfare Sub-Committees on Experimental Animal Licencing have granted the approval the use of experimental animals as described in the research methodology in the following research project:

"Phytochemical screening, toxicity study and antihypertensive activity evaluation of *Carpobrotus edulis*" (Ref Number 001/2019)

Yours sincerely

01/03/2019

Dr Samuel Swiswa
Chairperson: Departmental Ethics and Animal welfare Sub-committee (Experimental Animal Licencing)

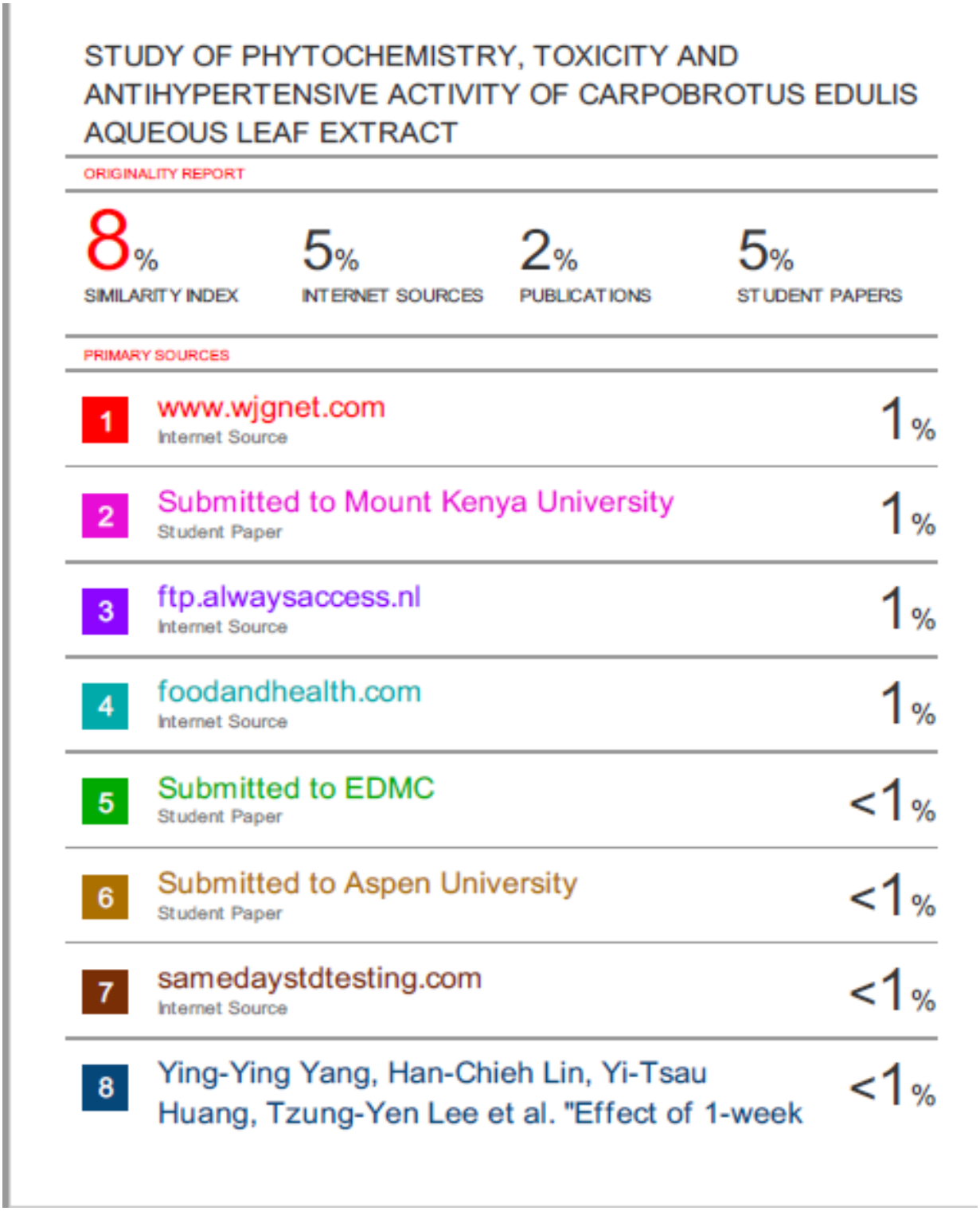
04/03/2019

Dr P.V Makaya
Deputy Director; Department of Veterinary Services
(Laboratory Diagnostics and Research)

01 MAR 2019

P.O. BOX CY 551, CAUSEWAY
ZIMBABWE

Appendix 9: Plagiarism Report



STUDY OF PHYTOCHEMISTRY, TOXICITY AND ANTIHYPERTENSIVE ACTIVITY OF CARPOBROTUS EDULIS AQUEOUS LEAF EXTRACT

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