"A COMPARATIVE STUDY OF THE ANTI-HYPERTENSIVE EFFECT OF *Aloe secundiflora* (ALOE), *Azadirachta indica* (NEEM) AND *Urtica dioica* (STINGING NETTLE) LEAF EXTRACT SYRUP ON NEW ZEALAND WHITE MALE RABBITS."

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DEDICATION

To my; husband Joseph, sons David, Steve and Jonathan, daughters Grace and Esther, brothers Newton and Daniel and, my mother Esther.

DECLARATION

This thesis is my original work and has never been presented for a degree in any other University.

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By Kamau Loice Njeri

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ABBREVIATIONS

MAP	-Mean Arterial Pressure
WHO	-Health Organization
NHLBI	-National Heart, Lung and Blood Institute
ACE	-Angiotensin Converting Enzyme
CNS	-Central Nervous System
ALE	-*A. secundiflora* syrup
SNLE	-*U. dioica* syrup
NLE	-*A. indica* syrup
FSD	-Furosemide
C.M.C	-Carboxymethyl cellulose

ABSTRACT

Herbal medicine has been extensively used across the globe dating back to the Ayurvendic medicine. Even though existing literature and practices show that *Aloe secundiflora* (Aloe), *Azadirachta indica* (neem) and *Urtica Dioica* (stinging nettle) are among the most commonly used plant extracts in the treatment of hypertension in powder or aqueous form, no comparative studies on their hypotensive effects had been done. In addition, literature on the extracts' significant dosage and associated physiological side effects was rare. Standardized method of extracting *A. indica* and *Urtica Dioica* syrups which are often distributed and administered in raw form was also not documented. In this study, 60% ethanol and water was used to produce standardized extracts of *A. indica* and *U. Dioica* leaves. Total extract yield of three stages and one stage and, cold and hot extraction was compared. Cold 60% ethanolic extracts of *A. indica* and *U. Dioica* powder and, dried *A. secundiflora* juice were formulated into syrup. The syrups were used to comparatively determine their hypotensive effect at doses of 10, 20, 40 and 80mg/kg on normotensive while 80mg/kg was used on 10% salt loaded unilaterally renal constricted and nephrectomized hypertensive male rabbits. In addition, their physiological side effects were also compared. Extraction using 60% three stage process produced significantly high total extract yield ($P < 0.0001$) both in *A. indica* 7.8g (15.5%) and *U. dioica* 5.8g (11.6%) than one stage 3.9g (7.8%) and 3.46g (6.92%) respectively. Hot extraction produced higher total extract yield ($P < 0.0001$) in both *A. indica* 10.6g (21.2%) 60% ethanolic extract and *U. dioica* 15.1g (30.2%) aqueous extract. These results suggest that use of hot three stages extraction process is the most efficient using 60% ethanol for *A. indica* and water for *U. dioica*. The lowest significant hypotensive dosage for *A. secundiflora* was 20mg/kg ($P < 0.0016$); it decreased mean arterial pressure (MAP) by 9.4mmHg (10.4%) and 16.02mmHg (17.6%) by the second and sixth hour respectively. *U. dioica* was significant at 40mg/kg ($P < 0.0001$) and decreased MAP by 15.1mmHg (18.3%) and 5.8mmHg (6.7%) by the second and sixth hour respectively while *A. indica* was significant at 40mg/kg ($P < 0.0001$) both at the second and sixth hour and decreased MAP by 12.2mmHg (13.9%) and 13.8mmHg (15.1%) respectively. All the three extracts showed significant anti-hypertensive effect on unilaterally nephrectomised hypertensive rabbits; *A. secundiflora* ($P < 0.005$), *A. indica* ($P < 0.039$) and *U. Dioica* ($P < 0.012$). They also significantly decreased MAP in renal constricted hypertensive rabbits; *A. secundiflora* ($P < 0.0001$), *A. indica* ($P < 0.0028$) and *U. Dioica* ($P < 0.0019$). *A. secundiflora* and *U. dioica* had significant diuretic effect ($P < 0.0001$) unlike *A. indica*. They decreased serum sodium ions ($q = 5.042$ and $q = 4.338$) respectively and chloride ions ($q=10.04$ and 6.792 respectively) but only *A. secundiflora* significantly decreased potassium ions ($q=6.731$) and showed laxative effect as well. The diuretic and laxative effects suggest why *A. secundiflora* demonstrated the highest hypotensive and anti-hypertensive effect while *A. indica* had the lowest. Both *A. indica* and *A. secundiflora* had significant hypothermic effect ($P = 0.034$ and $P = 0.023$ respectively) while *U. dioica* was not ($P = 1.735$). The study concluded that the most effective antihypertensive plant extracts with minimum physiological side effects was *U. dioica* followed by *A. indica* while *A. secundiflora* demonstrated the highest toxicity effect.

CHAPTER ONE: INTRODUCTION

1.1 Background information

High blood pressure (hypertension) is defined as sustained elevation of systolic blood pressure, diastolic blood pressure and mean arterial pressure (MAP) above 120mmHg, 90mmHg, and 110mmHg respectively (McClintic, 1985). Diastolic hypertension (high pressure in blood vessels) leads to arteriosclerosis (hardening, thickening and reduction in diameter of the vessels). Consequently, this results to insufficient blood supply to major body organs (ischemia) followed by deprivation of nutrients and eventual cell death (infarction) (Schmidt and Thews, 1989).

Hypertension overworks the heart leading to cardiac heart failure (myocardial insufficiency), heart attack and left ventricular hypertrophy (often associated with systolic hypertension). From clinical trials, left ventricular hypertrophy identifies individuals who require strict blood pressure control and risk factor modification (Salako, 1993). Systolic hypertension rises with age and is more frequent in individuals of over 60 years, women and the black population (Whitworth, 2003); since most of the black individuals are salt-sensitive as a result of inheriting hormones that control sodium excretion leading to hypertension (Freeman *et al.*, 2002). Systolic hypertension above 200mmHg may increase the danger of rupture of the blood vessels (cerebro-vascular accidents/stroke) (Kearney, 1996). According to Haslett *et al.*, (1999) on average people with uncontrolled high blood pressure are: seven times more likely to have a stroke, six times more likely to develop congestive heart failure and three times more likely to have a heart attack. On a similar vein, Whitworth, (2003) reports that

individuals with systolic hypertension are 4 times more likely to suffer from a stroke and 5 times to develop cardiac heart disease.

Currently, hypertension is the most prevalent cardiovascular disease in the world and has remained a major cause of hospitalization and death. According to Kearney *et al.,* (2005) it is estimated that by the year 2000, about 26% of the adult population had hypertension worldwide. In America, more than a third of persons aged 45 and above had been recorded to have high blood pressure (Hames and Greenlund, 1996). Furthermore, stroke was reported to be the third leading cause of mortality and accounted for over 163,000 deaths in 2001. This had high financial impact as a result of direct and indirect cost which in 2004 was predicted to be US$ 54billion (Whitworth, 2003). In Africa, the prevalence of hypertension ranges from 25% to 35% in adults aged 25-64 years and occurs across all socio-economic groups (WHO Report, 2002). According to a study carried out in Zambia, hypertension accounted for 12.3% and 2.8% of mortality rates in women and men respectively (Kafurabula, 1997). In Kenya, it is estimated that about 37% of the population is suffering from high blood pressure (Business Daily, 2011).

Even though systolic hypertension is a stronger independent predictor of cardiovascular morbidity and mortality it is less well controlled (34%) than diastolic hypertension (73%) among the hypertensive individuals (Kaufman *et al.,* 1996). Howeve, systolic hypertension can be controlled as espoused by Ezzati *et al.,* (2004) that within 3-5 years of effective lowering of blood pressure by 10 mmHg, most if not all of the increased risk of stroke is

eliminated. Therefore, treating and managing hypertension is undoubtedly one of the most clinically and cost effective cardiovascular interventions for primary and secondary prevention of stroke, coronary heart disease and diabetes related complications (Whitworth, 2003). Mild hypertension is usually managed by; maintaining a healthy weight, improved physical fitness, cutting back on alcoholic beverages, avoiding stress, not smoking, taking a diet rich in fruits and vegetables, fat free dairy foods and low sodium food. Persistent hypertension should be controlled by taking antihypertensive medicines either as conventional or traditional medicines (Kannel, 1996). As indicated in the United States Sixth Report of the Joint National Committee on high blood pressure, current conventional treatment for hypertension includes diuretics, α-agonists, β-blockers, calcium channel blockers, ACE (Angiotensin-converting enzyme) inhibitors, and angiotensin antagonists. These drugs can be used as monotherapy or in combination. However, they do not cure the disease but only relieve the symptoms, are frequently accompanied with side effects and are relatively expensive (Chobanian, 2003).

Even though lowering blood pressure can significantly reduce the possibility of the disease progressing to cardiovascular complication, its management requires strict adherence to the prescribed treatment. Unfortunately, most of the hypertensive individuals about 639 million from the developing countries are unable to comply with treatment due to the high cost of the drugs (Kearney *et al.*, 2005). The problem of compliance is compounded by the fact that most governments in the developing countries particularly in Africa rely on donation from the developed world. Therefore, they are unable to carry the burden of providing funds for

the treatment of most killer diseases such as hypertension whose related complications has caused many deaths. Stroke accounts for 50% and 51% deaths from hypertension related complications in men and women respectively in sub-Saharan Africa (ibid). This has necessitated the need to seek alternative strategies of treating hypertension to prevent new and recurrent stroke for instance, use of herbal medicine (Pobee, 1993).

Herbal medicine has been used for centuries, from Ayurvendic medicine to date (Klaus, 2004). Indeed, studies show that traditional healers in human and animal medicine fields use extracts of plant origin for the treatment of various illnesses and injuries because they are relatively cheaper and available to the local people than the allopathic drugs (Blumenthal *et al.*, 2000). According to Yokozawa (1996), western researchers are turning to traditional Chinese medicine for treatment of various diseases. There are different plant extracts that have been alleged to treat and manage hypertension including *A. secudiflora*, *A. indica* and *U. dioica*.

A. secundiflora is a member of Liliaceae/Asphodelaceae family and is associated with myth, magic and medicine since pre-biblical times (Chung *et al.*, 1996). According to Saleem, (2005) 0.1-3mg/kg lowered blood pressure in rats as an effect of a chemical substance called aloe emodin (Tobby, 1992). Atherton, (1997a) suggests a 100-200/day of the extract administration while in ayurvendic medicine the recommended dosage is 75-200 for 8-10 days. However, aloe juice is not recommended for oral use due to its strong laxative effect

(Blumenthal *et al.,* 2000). In addition, Robert and Davis, (1997) reported other physiological effects such as natriuresis, hypokalemia and diuresis.

A. indica is a member of family Meliaceae and was reported to reduce elevated heart rates and high blood pressure as an effect of its anti-histaminic and vasodilatory properties of nimbidin in aqueous leaf extracts (Ahmed and Grainge, 1986). Most of the documentation show that it has been used as an aqueous, methanolic or acetoneous extract intraperitoneally or intravenously administered to normotensive rats or cats but none to hypertensive animal. In addition, existing literature reported inconsistent effective dosage ranging from 5-1000gm/kg. Other physiological effects associated with *A. indica* include hypothermia (Okpanyi and Ezeukwu, 1981) and diuresis (Koley and Lal, 1994).

U. dioica is a member of family Urticaceae and was used from the time of the Roman Empire by soldiers during war with the British. Intravenous aqueous extract of *U. dioica* was reported to have no effect on blood pressure of rats whereas it produced a marked hypotensive effect by 31.7% and bradycardia in cats (Szentmiha'lyi, 1998) as an activity of high potassium level and xanthophylls (Trease and evans, 1978). Different effective dosages were reported ranging from 25mg/kg of aqueous extract to 6gm/ day of powder. Moreover, other physiological effects such as diuresis accompanied by sodium excretion (Tahri *et al.,* 2000) and hypothermia have been reported (Baraibar *et al.,* 1983).

Mostly, herbalists sell these plant materials particularly *A. Indica* and *U. dioica as* dried powder or aqueous decoction. As a result the dosage administered may be ineffective and inefficient for treatment of hypertension. The most documented extraction solvent was water while information about the nature of extraction; hot or cold was rare. In addition, use of alcoholic extract was rarely reported even though it is well documented that ethanol has both polar and non polar properties suitable to extract both ionic and organic substances. Therefore, there was need to extract the plant materials using 60% ethanol and further investigate their hypotensive activity and, effective dosage as this kind of research was not documented. In addition, there was need to determine the efficiency of hot and cold extraction using water and 60% ethanol as solvents. This facilitated the study to establish the most efficient solvent method of extracting standard and refined extracts of *A. Indica* and *U. dioica* leaf powder. However, the study did not investigate extract yield of *A. secundiflora* leaf using 60% ethanol and water since it is mostly used in form of dried or fresh Aloe juice and not leaf powder. Since most previous studies used aqueous extract, the study found it necessary to formulate the plant extracts to make them more palatable, control rate of absorption in the alimentary canal as well as preserve them.

Whereas from the literature it was clear that these plants possessed dose related hypotensive activity, the range between the lowest and highest dosages reported was so big. Consequently, the most effective hypotensive dosage was not evident; this necessitated further investigation to establish the minimum effective dosage. Moreover, investigations were on individual plants but none compared their hypotensive effect so as to determine

which possessed the highest activity. In addition, they used normotensive and rarely hypertensive animals; as such, there was need to determine both the hypotensive as well as the antihypertensive activity of these plant extracts. According to Guyton and Hall, (2000) experimental hypertension can be induced through unilateral nephrectomy and renal artery constriction accompanied with 10% salt loading. This develops into sustained hypertension as a result of increased blood volume caused by salt retention.

From literature, *A. indica and U. dioica leaves* had been administered as aqueous extracts through intravenous or intraperitoneal route to rats, cats or dogs. However, further investigation on the hypotensive effect of the extracts through the oral route was needed as it is currently the easiest and convenient method of administering drugs (Simler *et al.*, 2008). In this study rabbits were chosen as the most appropriate experimental animal as they are easy to handle, administer the extract and measure blood pressure using electronic blood pressure machine without anaesthetizing. Moreover, literature in relation to the hypotensive effect of these plants using rabbits was rare.

From previous studies, *A. secundiflora, A. indica* and *U. dioica* leaf extracts possess diuretic, hypothermic and laxative effect (Atherton, 1997b). According to Tierney *et al.*, (2001), such medicinal herbs decrease serum electrolytes and eventually affect the cardiovascular system (Ericksson *et al.*, 1999) such as; vasoconstriction, cardiac arrhythmia and bradycardia (Polderman, 2009; Kudenchuk, 1999). That explains why most governments and health authorities discourage use of herbal remedies (Akpanabiatu *et al.*, 2005). For instance, in

2002, the U.S. Food and Drug Administration (FDA) required manufacturers of aloe-containing laxative to remove those products from the market because no credible evidence showed aloe laxative to be either safe or effective for use (Blumenthal *et al.*, 2000). This necessitated the study to investigate other physiological activities of *A. secundiflora* and, 60% ethanolic extract of *A. indica* and *U. dioica*. Moreover, there was need to compare their effects at a given dosage and particularly in relation to blood pressure.

1.2 Statement of the problem

Hypertension remains a major cause of hospitalization and death in the world; it is the commonest cardiovascular disease in Africans. Compliance with treatment prevents the likelihood of hypertension progressing to more severe form. Although the cost of drug is the most important determinant of compliance, treatment is beyond the budgetary capabilities of most developing countries. As a result, herbal medicine has been an alternative and affordable treatment. *A. secundiflora*, *A. indica* and *U. dioica* leaves are among some of allegedly antihypertensive plant materials currently sold in the market at a relatively lower cost.

However, they are usually administered by people who have limited clinical knowledge and understanding about their effective antihypertensive oral dosage. The existing literature report inconsistent effective hypotensive dosage ranging from 75-200mg/day of *A. secundiflora* orally administered, 5-1000mg aqueous solution of *A. indica* and 25mg/kg aqueous extract to 6gm powder of *U. dioica* intravenously given. Moreover, different experimental animals produced varied effects for example *A. dioica* was significant in cats

but was not in rats. In addition the herbalists have limited knowledge of other potential physiological effects caused by these plant materials especially when administered concurrently or in addition to conventional drugs. The plant materials are mostly sold in raw form and literature on the most efficient standard method of extraction is rare. This study sought to bridge these gaps in order to make use of herbal medicine more meaningful in the treatment of the dreadful hypertensive conditions.

1.3 Purpose of the study

The purpose of the study was to; comparatively determine the antihypertensive activity, effective dosage and other physiological effects of standard plant extracts of *A. secundiflora* and, cold 60% ethanolic extract of *A. indica* and *U. dioica* leaves formulated into syrup and orally administered to New Zealand white male rabbits. In addition, the most efficient method of producing standard plant extracts was determined.

1.4 Objectives

Specific objectives

i. To determine and compare total extract yield of *A. indica* and *U. dioica* leaf in a three-stage extraction process using cold 60% ethanol and, the effect of temperature using cold and hot solvent (60% ethanol and water).

ii. To determine and compare the hypotensive effect of *A.secundiflora, A. indica* and *U. dioica* leaves extract syrup on both normotensive and hypertensive New Zealand White male rabbits.

iii. To determine and compare the diuretic effect of *A. secundiflora, A. indica* and *U. dioica* leaf extract in New Zealand white male rabbits.

iv. To determine and compare the effect of *A. secundiflora, A. indica* and *U. dioica* leaves extract on serum electrolytes in New Zealand white male rabbits.

v. To compare the observable laxative effect of *A. secundiflora, A. indica* and *U. dioica* leaves extract in New Zealand white male rabbits.

vi. To determine and compare the hypothermic effect of *A. secundiflora, A. indica* and *U. dioica* leaves extract in New Zealand white male rabbits.

1.5 Hypotheses

i. Three stages extraction process using hot solvent produces higher extract yields than one stage and cold extraction using *A. indica* and *U.dioica* leaves.

ii. *A. secundiflora, A. indica* and *U. dioica* leaf extract syrup has hypotensive and anti-hypertensive effect on normotensive and hypertensive male rabbits respectively.

iii. *A. secundiflora, A. indica* and *U. dioica* leaf extract syrup has diuretic effect on normal male rabbits.

iv. *A. secundiflora, A. indica* and *U. dioica* leaf extract syrup decreases serum electrolytes of normal male rabbits.

v. *A. secundiflora, A. indica* and *U. dioica* leaf extract syrup has laxative effect on normal male rabbits.

vi. *A. secundiflora, A. indica* and *U. dioica* leaf extract syrup has hypothermic effect on normal male rabbits.

1.6 Justification

Although literature on hypotensive activity of *A. secundiflora, A. indica* and *U. dioica* leaf extracts has been documented, information on their antihypertensive effect is rare. Particularly, no study has been done to compare their effective antihypertensive dosage and associated physiological effects. In addition, information on the most efficient method of extraction of *A. indica* and *U. dioica* leaves which are often administered in raw form is scantly documented while studies using rabbits were uncommon. Therefore, this study was very timely because it came at a time when use of herbal medicine in the treatment of various conditions including high blood pressure has taken a centre stage.

1.7 Operational definition of key terms

i. Antihypertensive effect: Potential of an extract syrup to reduce high blood pressure in rabbits

ii. Antihypotensive effect: Potential of an extract syrup to reduce normal blood pressure in rabbits

iii. Diuresis: Excessive removal of water through urine

iv. Hypothermic effect: Potential of an extract syrup to reduce normal temperature in rabbits

v. Laxative effect: Frequent removal of faeces together with water

vi. Renal constriction: Ligation of the renal artery of the male rabbits in order to limit normal blood flow

vii. Unilateral nephrectomy: Removal of one of the kidneys from the male rabbits

CHAPTER TWO: LITERATURE REVIEW

2.1 Hypertension

Blood pressure is a measure of the force exerted by circulating blood on the walls of the main arteries (WHO,1999). Systolic blood pressure is the highest pressure created by the contraction of the heart and is about 120mmHg and diastolic blood pressure is the lowest pressure in the blood vessels as the heart fills with blood and is about 70mmHg in a healthy young adult. Arterial pressure is conventionally written as systolic pressure over diastolic pressure, for example, 120/80. The mean arterial pressure (MAP) is the average pressure throughout the cardiovascular system and equals the diastolic pressure plus one-third of the pulse pressure, about 110mmHg in a young adult (Ganong, 2003).

High blood pressure or hypertension is a medical condition where the mean arterial pressure is chronically elevated above the normal (Guyton and Hall, 2000). A person whose MAP, diastolic blood pressure and systolic blood pressure are greater than 110mmhg, 90mmHg and120mmHg respectively is considered to be hypertensive. In severe hypertension, the MAP can rise from 150-170mmHg, with diastolic blood pressure as high as 110mmhg and systolic blood pressure occasionally as high as 250mmHg (Kannel, 1996). According to the recommendations of the World Health Organization (WHO), hypertension is regarded as systolic and diastolic blood pressure above 160mmHg and 95mmHg respectively (Schmidt and Thews, 1989). According to NHLBI (Chobanian, A. V. 2003) blood pressure is classified into three categories of adults aged 18 and over as:

Category	Systolic (mmHg)	Diastolic (mmHg)
Normal	130 or Less	85 or Less
High normal	130-139	85-89
High blood pressure	140 or More	90 or more

Table 1 Blood pressure classification according to NHLBI

If arterial pressure is too low, the blood may not reach certain areas of the body while when it is too high, the heart and blood vessel walls may be damaged (Schmidt and Thews, 1989). At severely high mean arterial pressure of about 50% or more above the normal, a person can only live for a few years unless appropriately treated (Guyton and Hall, 2000). However, even moderate elevation of the arterial pressure leads to shortened life expectancy (Chobanian, 2003). In addition, it may progress to malignant hypertension (accelerated hypertension) and this can lead to serious life-threatening complications, such as:

i) Left ventricular hypertrophy, coronary heart disease and consequently heart attack (MacGregor, 1997). This view is corroborated by a study done in Nigeria which revealed that 95% of the individuals who sought for renal complications treatment had left ventricular hypertrophy (Ifeuma *et al.*, 2006).

ii) Stroke which lead to paralysis, dementia, blindness and multiple other serious disorders (Law *et al.*, 2003). The risk of stroke (cerebrovascular event) rises with increasing blood pressure, such that a 5mm Hg decrease in diastolic blood pressure provides 34% reduction in stroke risk (Law *et al.*, 2003).

iii) Multiple hemorrhages in the kidneys which may cause kidney failure, anemia and death (Guyton and Hall, 2000). In USA, approximately 70% of kidney patients are hypertensive (Chobanian *et al.*, 2003).

In clinical practice there are two types of hypertension; primary (essential) hypertension and secondary (systemic) hypertension. Primary essential hypertension has no specific cause and makes up about 90% of all hypertensive patients (Guyton and Hall, 2000). If a cause of hypertension can be determined, the hypertension is designated as secondary hypertension and occurs in 10% of all hypertensive cases (Schmidt and Thews, 1989). The main causes of secondary hypertension is body mass index, stress, arteriosclerosis, smoking, contraceptives in females, high salt diet, excess alcohol consumption, sedentary lifestyle, genetics, age, sex, ethnic groups, high blood cholesterol, diabetes mellitus, pre-existing vascular diseases, Cushing syndrome, hyperthyroidism and acromegaly (Lorenzo *et al.*, 2002).

There are two types of secondary hypertension that is; systolic and diastolic hypertension. Systolic hypertension is caused by increased cardiac output and rigidity of the walls of the aorta and main arteries (arteriosclerosis) while secondary diastolic hypertension results from vascular damage such as atherosclerosis and smooth muscle peripheral constriction. This leads to diminished blood supply (ischemia) to organs such as kidney, liver, pancrease, brain and retina. Eventually the organs undergo degenerative changes due to lack of nutrients known as infarction (Schmidt and Thews, 1989). About 25% of secondary hypertension is renal hypertension, and is caused by increased release of rennin,, aldosterone (ibid),

pheochromocytoma (Delgado and Remers, 1998), nephritis, renal artery thrombosis, renal artery infarctions and renal artery stenosis (Haslett *et al.,* 1999). In renal (rennin-dependent) hypertension; angiotensin 11 induces release of intracellular calcium via protein kinase C (Inositol triphosphate) mechanisms (Ganong, 2003).

About 40 to 60% essential hypertension is genetic (Lorenzo *et al.*, 2002). Consequently, hormones that regulate sodium excretion in NaCl-sensitive individuals are inherited leading to high blood pressure in Caucasians, African-Americans and diabetic populations (Freeman *et al.*, 2002). This accounts for about 30-50% of hypertensive persons and also a small percentage of non hypertensive persons (Luft and Weinberger, 1997). According to salt hypothesis, high salt intake in the diet by salt-sensitive individuals increases blood pressure and the risk of cardiovascular disease (Kaufman *et al.,* 1999). On the same vein, Freeman *et al.*, (2002) has reported that addition of NaCl (5, 10 and then 15g /day) to the chimpanzee usual diet for 84 weeks increased mean systolic and diastolic blood pressure by 33mmHg and 10mmHg respectively. According to Guyton and hall, (2000) high sodium chloride level in the extracellular fluid causes sustained hypertenssion due to increased peripheral resistance that result from increased blood volume.

The kidney plays a major role in regulating the amount of sodium chloride in the body and consequently blood pressure. Therefore, any changes in its anatomy may affect the cardiovascular system significantly. For instance, in clinical practice, renovascular hypertension is caused by atherosclerosis of the renal artery (Ganong, 2003) resulting in

>80% luminal narrowing (severe renal artery stenosis). As a result there is increased salt retention that leads to persistent significant hypertension (Plouin *et al.,* 1998 and Mailloux *et al.*, 1994). Renovascular hypertension accounts for 1-10% of 50 million people in the United States and is more common among whites (27-45%) than Africans (8-19%) but it is also common among the elderly population over 70 years (62%). Moreover, excess aldosterone secreted in primary aldosteronism condition causes the same effect (Guyton & Hall, 2000). Consequently, in the human being, the body-fluid system is the fundamental basis of long term arterial pressure control (Kannel, 1996).

Therefore, sustained or severe renal hypertension can be produced experimentally by constricting one renal artery with the other kidney intact ("Two kidney" Goldblatt hypertension) and unilateral nepherectomy with 10% salt loading hypertension. This increases circulating rennin which consequently increases the secretion of aldosterone, facilitates release of norepinephrine by a direct action on postganglionic sympathetic neurons and causes renal tubules to increase Na^+ reabsorption. This leads to increased extra cellular fluid that develops to sustained hypertension known as 'two kidney" Goldblatt and, renal hypertension respectively (Guyton & Hall, 2000).

2.2 Epidemiology

Hypertension is by far the most prevalent cardiovascular disease in the world. Data from the World Health Organization (WHO) in the year 2000 estimated that nearly one billion people or ~26% of the adult population had hypertension worldwide. It was common in both

developed (333 million) and undeveloped (639 million) countries. It also causes an estimated 7.1 million deaths a year or approximately 13% of total mortality. In 2000, about 62% of strokes were attributable to systolic blood pressure greater than 115 mm Hg. In sub-Saharan Africa, deaths were 73,000 in men (50%) and 107,000 in women (51%) and a total disability of 2.6 million (Kearney *et al.*, 2005).

It is estimated that more than a third of Americans aged 45 or older have high blood pressure and among them, more than 50% are aged 60 or older (Hames and Greenlund, 1996). Based on NHLBI report, high blood pressure tends to be more common, occurs at earlier age, and is more severe for many Africans Americans; for instance, out of three African Americans one has high blood pressure. Furthermore, African Americans are at higher risk for hypertension than any other race or ethnic group because most are genetically salt-sensitive (Chobanian, 2003). Males are more likely to develop high blood pressure than women in young adulthood and early middle age. Thereafter, blood pressure tends to rise as people grow older; that is, in both men and women (Pobee, 1993).

A study done in Zambia revealed that, the total males admitted due to hypertension were 70 while females were 242, i.e. about 1:3 male to female ratio at admission. The case mortality rates were women 12.3% and men 22.8% indicating that males had a higher mortality rate than women. This study also revealed that males came in rarely but when they did, they came in with worse disease than females. By age distribution, 50% of male patients were below 35

years (youth) and 50% were above 35 years. In females 25% were below 35 years of age (youth) and 75% were above 35 years of age (Kafurabula, 1997).

From about 25 years ago, high blood pressure became established in Kenya (Lore, 1993). According to a survey done in Kenya between 1984 and 1987, the prevalence of systolic blood pressure equal or above 160 and /or diastolic blood pressure 95mmHg or on antihypertensive medication for ages 20-59 were 2.2% in males and 1.2% in females. However, in both sexes individuals of ages 50-59 were the most affected, males 9.5% and 4.2% in females (The Intersalt Cooperative Research Group, 1989). This data indicate that, hypertension in Kenya was largely age dependent and the most affected groups were males (Jenson *et al.* 2010). Currently, about 37 per cent of the population is suffering from high blood pressure followed by heart disease at 12.7 per cent (Business Daily, 2011). It is therefore clear that hypertension should be treated and controlled promptly upon diagnosis.

2.3 Diagnosing hypertension

Atherosclerotic plaque which is a major cause of hypertension is diagnosed by ultrasound and appears as discrete areas of thickening of the arterial wall (Polak, 2001). In addition, high blood pressure may be diagnosed by use of an ECG (Electrocardiogram), apex cardiogram, stethoscope, phonocardiography, medical imaging, echocardiogram, angiocardiography and right heart catheterization (Schmidt and Thews, 1989). Currently, digital blood pressure machines are also in use (Chobanian, 2003).

2.4 Prevention, treatment and management of hypertension

2.4.1. Ayurvedic approach

From ayurvedic perspective, hypertension is treated depending on what type of hypertension has been diagnosed. Vata hypertension is associated with nervous system disorders and keeps on fluctuating; it is treated by eating an entire clove of garlic. Kapha hypertension is related with obesity, edema and high cholesterol and remains continually high. It is treated by avoiding foods rich in fat and cholesterol and, by eating plenty of onions and garlic. Pitta hypertension is associated with anger, stress and liver disorders; it is treated with aloe gel, which is believed to calm the nerves and relieves heat and stress (*Ayurveda pharmacopoeia*, 1979).

2.4.2 Conventional drug approach in management of hypertension

Therapy using antihypertensive agents evolved rapidly between 1950 and 1960. There are various conventional drugs used in the treatment and management of hypertension. Treatment is based on the type, cause, phase of hypertension and age of the patient (Delgado and Remers, 1998). Diuretics are effective antihypertensive treatment suitable for a range of different patient groups, especially the elderly, those at increased risk of stroke and patients with evidence of target organ damage such as left ventricular hypertrophy or proteinuria. Diuretics reduce blood volume by increasing the rate of urine formation by inhibiting sodium transport along the nephrone. They are such as; Bendrofluazide, Furosemide and Torsemide ((Delgado and Remers, 1998; Kimotho *et al.*, 2004). ACE (angiotensin-converting-enzyme) inhibitors are used as inhibitors of the rennin-angiotensin system e.g Trandolapril (Delgado and Remers, 1998 and Kimotho *et al.*, 2003).

Calcium channel blockers act by decreasing calcium levels in vascular tissues and consequent vasodilation (Schmidt and Thews, 1989) that results in reduction in peripheral vascular resistance. An example of such drugs is Amlodipine besylate (Delgado and Remers, 1998). Alpha-receptor agonists for example Clonidine, are widely used medications and act by stimulating the α-adrenergic receptors which in the CNS reduces sympathetic outflow to the cardiovascular system, this produces a hypotensive effect (Kimotho *et al.,* 2003).

Vasodilators interfere with Ca^{2+} entry and release from intracellular stores and activation of $_{c}$GMP. This causes a decrease in peripheral resistance and thus increased blood flow as a result relaxing the smooth muscles. Example of such drugs are; Sodium nitroprusside and Hydralazine hydrochloride (Delgado and Remers, 1998 and Haslett *et al,* 1999). Potassium channel agonists activate ATP-sensitive potassium channels decreasing intracellular Ca^{2+}. This reduces the excitability of smooth muscles and as a result open potassium channels in the plasma membrane of vascular smooth muscle. An efflux of potassium from the cell follows, resulting in hyper-polarization of the membrane. As a result membrane excitation is inhibited leading to vasodilatation. These drugs are also known as "Potassium channel openers"; examples are Diozoxide and Minoxidil (Delgado and Remers, 1998).

ß-blockers such as Atenolol or Propranolol have been used across the cardiovascular disease spectrum and still continues to expand since its introduction more than 50 years ago (Messerli and Grossman, 2004). They are used to treat blood pressure, angina pectoris and myocardial infarctions (Gheorghiade and Fonarow, 2004), tachycardia and hypertension due

to alcoholism (Delgado and Remers, 1998). They act by depressing the sympathetic nervous system through the β-adrenergic receptors (Haslett *et al.,* 1999). Consequently, reduce oxygen demand by reducing heart rate/negative chronotropic action and blood volume by reducing renin secretion (Delgado and Remers, 1998).

Anti-arrhythmia drugs such as Amiadazoline act by prolonging myocardial cell action (Kimotho *et al.*, 2003). HMG-CoA (3-hydroxy-3 methylglutaryl Coenzyme A) reductase inhibitors such as Lovastatin are used to treat hypertension caused by atherosclerosis that is caused by high levels of cholesterol a condition known as hypercholesterolemia (Kimotho *et al.,* 2003).

2.4.3 Herbal medicine in the treatment and management of hypertension

2.4.3.1 *A. secundiflora* (Aloe)

Botanical description

Scientific name: *Aloe secundiflora*

Family: Asphodelaceae / Liliaceae

Synonym: Aloe floramaculata, Aloe marsabitensis, or Aloe engleri.

Common names: first aid plant, aloe latex, burn plant elephant's gall, lily of the desert, miracle plant, plant of immortality and medicine plant (Chung *et al.,* 1996).

A. secundiflora species plant is a large xerophyte, succulent evergreen perennial plant. It grows up to 1.5 meters in height with a large stem supporting a rosette of narrow lanceolate leaves up to 60cm long. The leaves are whitish green on both sides and bear spiny teeth on

the margins. For commercial use, several of the larger leaves are taken from each plant every few weeks. The scarlet (dark red) drooping flowers grow in a long raceme at the top of the flower stalk about 40cm high. The fruit is a triangular capsule containing numerous seeds (Fig. 1). The main aloe species in Kenya are *Aloe secundiflora*, *Aloe turkanensis* and *Aloe kilifi*. *A. secundiflora* is commonly found in Maral Samburu County (Waihenya *et al.*, 2002).

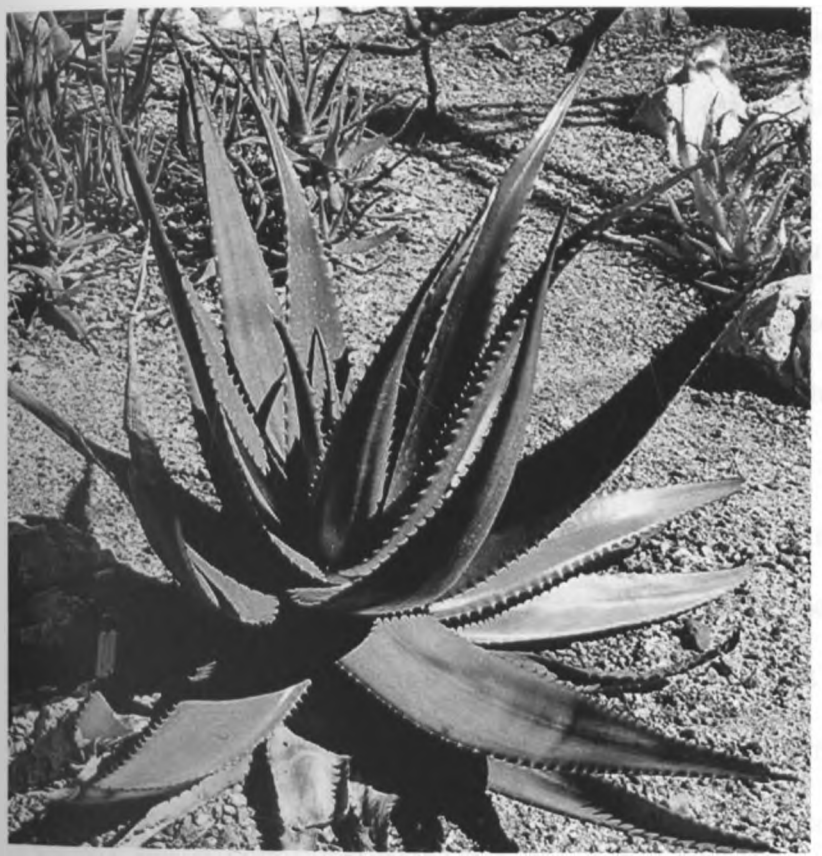

Figure 1 *Aloe secundiflora* (Aloe)

Pharmacological activity of *A. secundiflora*

The first reference to Aloe species in English was a translation by John Goodyew in A.D 1655 of Dioscorides medical treatise De material medica which he wrote in AD 70-90. Traders first brought Aloe Vera to London in 1693 and by 1843; considerable amounts were being imported to be made up into medicine (Trease and Evans, 1978). It is currently a common ingredient in cosmetics and hand lotions. 'The International Aloe Science Council' which is charged to certificate the purity of Aloe has established standards for aloe leaves and aloe gel (Tobby, 1992). *Aloe sp.* has been associated with myth, magic and medicine since pre-biblical times. Legends state that pharaoh, and the royal family of Egypt, kept aloe as a palace plant assigning it a very high status. It is suggested that the Egyptian queens Nefertiti and Cleopatra used it as part of their regular beauty regimes.

Alexander the great in 333 B.C was persuaded by his mentor Aristotle to capture the island of Socotra in the Indian Ocean for its famed Aloe supplies needed to treat his wounded soldiers (Renolds, 1985). Aloe is also mentioned in the Bible (St John's gospel) but this was in fact Lignin Aloe and not true Aloe lignin. Lignin Aloe is a tree whose scented bark was used for incense as well as an ingredient in embalming the dead. The medicinal values of Aloe herb have been known for centuries for instance, a 3500-years old document (papyrus Ebers) at the Leipzing University describes Aloe and its values as a medicine (Chung *et al.*, 1996).

Aloe juice has also been effective in lowering blood pressure and may also promote the formation of new blood vessels in damaged tissue (Tobby, 1992). Experiments show that when Aloe juice is ingested, aloe-emodin (the quinone aglycone) is released and these may produce acceleration of ethanol metabolism rate in vivo by 40 %. Furthermore, studies show that aloe-emodin has dose-dependent hypotensive effect when administered at doses of 0.1, 1, and 3mg/kg in rats (Saleem *et al.*, 2005).

Aloe also treats coronary heart disease, anxiety disorders, aortic aneurysm, chronic fatigue syndrome which is all related to hypertension (Winters *et al.*, 1981). It has been suggested thatloe juice lowers blood pressure by dilating the capillaries, thereby increasing blood circulation. According Waihenya *et al.*, 2002 acemannan present in *A. secundiflora* stimulates production of nitric oxide leading to vasodilation (Delgado and Remers, 1998). Frequent use of *A. secundiflora* influences fluid and electrolyte loss from body tissues and, hypokalemia (Robert and Davis, 1997). It also stimulates peristalsis, motility of the colon and active chloride secretions (Reynolds and Dweck, 1999).

Few studies have been performed to assess the laxative effect in humans (Ishii *et al.*, 1998). Anthraquinone derivatives (aloin A and aloin B) present in aloe juice (Robert and Davis, 1997) possess very powerful purgative (laxative) action and are used in treatment of constipation (Herlihy, 1998). As such, Aloe should be taken for a maximum of 8-10 days at a dose of 75, 100, and 200mg dosage per day (Lourdes *et al.*, 2008). However, Atherton,

(1997a) suggested 100-200mg of aloe juice taken in the evening while Lourdes *et al.*, (2008) recommended 50-300mg given that it produced laxative effect.

From the literature, precise information on Aloe juice dosage was lacking while major side effects had not been well established. This necessitated further study to establish the lowest significant hypotensive dosage (Atherton, 1997b) and other toxicological effects so as to determine its safety as a potential antihypertensive plant extract. *A. secundiflora* is one of the most common and abundant *Aloe sp* in Kenya however, there was rare documentation about its hypotensive activity and was therefore chosen for this study.

2.4.3.2 *A. indica* (Neem)

Botanical description

The scientific name: *Azadirachta indica*

Family: Meliaceae / mahogany

Synonym: *Melia azederach*

Common names: nim, margosa tree, bead tree, holy tree, and Indian lilac tree (Subapriya and Nagini, 2005).

A. indica tree is an evergreen woody plant. Mature trees attain heights of 7-20m with a spread of 5-10m. The boles are short and stout with a rough dark bark. They have alternating compound leaves each comprising of 5-15 leaflets arranged in alternate pairs with a terminal leaflet. The small white flowers that produce a very sweet jasmine-like scent are born in

maxillary clusters. The fruit is a smooth, ellipsoidal drupe about 2cm long (Ahmed and Grainge, 1986) (Fig 2). In Kenya, it is commonly found in Kilifi.

Pharmacological activity of *A. indica*

The centuries-old healing system known as Ayurvedic medicine has utilized these timeless *A.indica* formulations as an important part of the Ayurvedic pharmacy. The empirical basics of the therapeutical use of the species have been laid down in classical texts of Ayurveda. The first recorded medicinal uses of neem are attributed to an ancient Indian culture over 4500 years ago and were expressed in the oldest Sanskrit writings. In traditional India the *A. indica* tree has been revered as the "village pharmacy" because for centuries, the whole tree has been used for medicinal purposes (Klaus, 2004). Historically, *A. indica* leaves have been the primary neem ingredients in the ancient medicinal preparations because of their availability throughout the year and the ease of extracting the compounds (Ahmed and Grainge, 1986).

A. indica is considered the most powerful blood purifier and detoxifier in Ayurvedic usage (Subapriya and Nagini, 2005). Intravenous aqueous *A. indica* leaf extract has been reported to cause immediate and sustained dose-dependent fall in blood pressure in anaesthetized dogs (Klaus, 2004). The same effect has been recorded in rats given 100, 300 and 1000mg/kg of *A. indica* leaf extracts intravenously (Koley and Lal, 1994). In addition intravenous administration of aqueous leaf extract at doses of 5-200mg/kg induced profound dose-

dependent hypotension in rabbits as an effect of nimbidine in *A. indica* leaf extract which caused vasodilation and brandycardia. Moreover, an intravenous dose of 40mg/kg of the extract was shown to exhibit anti-arrhythmic activity against ouabain-induced dysrhythmia in rabbits (Ahmed and Grainge, 1986).

Figure 2 *A. indica* (neem)

It was also reported to cause side effects for example; methanolic leaf extract displays a low oral LD_{50} of about 13g/kg in an acute toxicity test in mice. Another effect was hypothermia (Okpanyi and Ezeukwu, 1981), for instance, acetoneous leaf extract induced a dose-dependent hypothermia in doses of 50, 100 and 200mg/kg (Pillai and Santhakumari, 1984). *A. indica* leaf extract sodium nimbidinate was reported to cause diuresis (Koley and Lal, 1994) while some studies reported that *A. indica* leaf extract caused reduced or no urine productions (Van der Nat *et al.*, 1991).

According to Willcox *et al.*, (2004) *A. indica* leaf extract is effective in only small doses and has low toxicity. However, there was need to do further investigation on hypotensive effect of ethanolic leaf extract and the lowest significant dosage orally administered in male rabbits as this had not been done before. It was also important to determine other physiological activities such as; diuresis, laxative, hypothermia and effect on serum electrolyte level in relation to its activity blood pressure.

2.4.3.3 *U. dioica* (stinging nettle)

Botanical description

Scientific name: *Urtica dioica*

Family: Urticaceae

Synonyms: *Urtica gracilis, Urticae radix*

Common names: Stinging nettle, common nettle, nettle, nettle leaf, Chinese nettle, common stinging nettle, great nettle and great stinging nettle (Adamski and Bieganska, 1984).

The most prominent member of the genus is *U. dioica* (Tita *et al.*, 1993). It has a rather stout hollow stem and the plant grows 2-4 feet tall. They have somewhat oval dark green leaves with a long-stalk; heart-shaped base, serrated margin and a pointed tip (Fig 3). The plants are covered with sharp stinging hairs whose small cells at the swollen base are filled with an acid fluid (venom) that causes an intense skin irritation (inflammation). They are dioecious because the male and female flowers grow on separate plants (Adamski and Bieganska, 1984). In Kenya, *U. dioica* occurs mainly in Molo.

Figure 3 *U. dioica* (stinging nettle)

The most prominent member of the genus is *U. dioica* (Tita *et al.*, 1993). It has a rather stout hollow stem and the plant grows 2-4 feet tall. They have somewhat oval dark green leaves with a long-stalk; heart-shaped base, serrated margin and a pointed tip (Fig 3). The plants are

covered with sharp stinging hairs whose small cells at the swollen base are filled with an acid fluid (venom) that causes an intense skin irritation (inflammation). They are dioecious because the male and female flowers grow on separate plants (Adamski and Bieganska, 1984). In Kenya, *U. dioica* occurs mainly in Molo.

Pharmacological activity of *U. dioica*

U. dioica has a long history of medicinal use. The Romans used to rub the leaves on their bodies to restore circulation to limbs numbed by the British winters (Tita *et al.,* 1993). Perfusion of dry aqueous extract from *U. dioica* reduced arterial blood pressure by 15% at 4mg/kg/hr and 38% at 24mg/kg/hr accompanied by diuretic and natriuretic effects (Szentmiha'lyi, 1998). In addition, a dose of 26.6mg/kg aqueous extract of *U. dioica* given by canulla to cats produced marked hypotensive effect and bradycardia while 25mg/kg it demonstrated rapid decrease of blood pressure (31.7%) in rats (Tahri *et al.*, 2000).

However, earlier studies reported no effect on blood pressure of rats but did demonstrate immediate hypotension in cats. It also lowered the body temperature of rats (Baraibar *et al.*, 1983). Moreover, other animal studies had shown a decrease in blood pressure from nettle aerial parts but the effect was slight and inconsistent accompanied by diuretic, hypothermia and sodium excretion (Tahri *et al.,* 2000). According to Szentmihlyi, (1998) *U. dioica* leaf extract lowered blood pressure by its vasodilatory activity. Since atropine had no effect on bradycardia actions of *U. dioica* leaf extract, a mode of action via alpha-adrenoreceptors was suggested (Tahri *et al;* 2002). Xanthophyll present in *U. dioica* leaf extract was reported to

have vasodilatory activity (Trease and Evans, 1978) while its CNS-depressant activity lowered body temperature in rats. In view of the documented pharmacological actions of *U. dioica* leaf extract, excessive use interacts with current therapy of high or low blood pressure and potentates drugs with CNS depressant actions (Szentmiha'lyi, 1998).

According to Tita *et al.,* (1993) *U. dioica* was historically used as a mild diuretic. However, Szentmihalyi, (1998) reported that no significant diuretic effect was observed during 2 hours after oral administration of aqueous extract 1gm/kg neither did ethanolic extract. But urinary excretion increased significantly after intraperitoneal administration of 500mg/kg. The diuretic activity was caused by flavonoids and the high potassium: sodium ratio content of aqueous extract which corresponded to 448:1 (ibid).

Furthermore, several uncontrolled trials have reported improvements in urological symptoms compared with baseline values following administration of *U. dioica* root extract 600-1200 mg daily for three weeks to 20 months. This resulted in greater improvements in urinary flow, urine volume and residual flow. By day two and throughout the study, there was a significant increase in urine volume by 9.2% ($p < 0.0005$) observed daily in 32 patients with myocardial or chronic venous insufficiency treated with 15ml of nettle juice three times daily for two weeks (Schneider and Rubben, 2004).

According to Riehemann, (1999) an ethanol extract of *U. dioica* (plant part unspecified) showed low toxicity in rats and mice after oral and intraperitoneal administration at doses equivalent to 2 g/kg. However, Chubrasik *et al.,* (2009) recommended daily dosage as 4-

8g/day of powder or aqueous extract (1:1) taken as 2-5ml three times a day. From the documented findings, there were inconsistent reports about the effective hypotensive dosage of *U. dioica* while literature on other physiological effects of *U. dioica* such as hypothermia, serum electrolyte level and laxative activity was rare. In addition, most research work had been done using aqueous extract on cats, rats or dogs administered intraperitoneally or intravenously. It was therefore necessary to do further investigation using ethanolic extract orally administered to rabbits.

2.5 Physiological effects of antihypertensive herbal medicines on the cardiovascular system

2.5.1 Diuretic, laxative and serum electrolyte effects

Blood volume and serum electrolyte levels determine blood pressure and are affected by the amount of water in the body and electrolytes excreted (Grobee, 1984). Diuretics such as Furosemide are clinically used to treat volume-loading hypertension. They reduce blood pressure by decreasing extra cellular fluids. This leads to loss of serum electrolytes such as sodium and potassium (Guyton and Hall, 2000). Similar effects are caused by some antihypertensive herbal medicines which possess either or both diuretic and laxative activity (Tierney *et al.*, 2001). Consequently, over dosage or long term use of these drugs/herbal medicines may lead to excessive loss of body fluids and serum electrolytes (Ericksson *et al.*, 1999). Since these ions play an important role in cardiovascular activity, severe electrolyte disturbances may lead to life-threatening consequences such as heart failure, shock, coma, or tetany (Tierney *et al.*, 2001). Consequently, serum electrolytes should be investigated in

hypertensive patients on medication (Nurminen *et al.,* 1998). A test for electrolytes includes the measurement of sodium, potassium and chloride ions (Grobee, 1984).

Potassium ion is in an intra-cellular fluid cation and has been reported to be among the protective electrolytes against hypertension (Nurminen *et al.,* 1998). Potassium ion channels are highly selective and are crucial for the hyperpolarization of body cells such as neurons, after an action potential has been fired (Pierdomenico, 2009).

The reference range for potassium ion is 3.6-5.0 mmol/l. Potassium ion values below 3.0mmol/l (hypokalemia) occur as a result of either a low intake over a period of time or an increased loss of potassium through vomiting, diarrhea and long-term therapy with diuretics(Tierney *et al.,* 2001). The fluids of the gastrointestinal tract contain relatively high concentrations of potassium and their removal or loss through use of laxatives can produce serious deficit (Bishop, 2000). Hypokalemia increases muscle irritability and can cause cessation of the heartbeat in systole (contraction) associated with arrhythmia, tachycardia and cardiac arrest. In severe cases it leads to muscle weakness, paralytic ileus, decreased reflex response and respiratory paralysis (Pierdomenico, 2009).

Values above 6.0mmol/l (hyperkalemia) occur when there is a decreased output of urine with normal intake of potassium or excessive intake of potassium (Bishop, 2000). This can seriously inhibit muscle irritability including the heart to the point of; paralysis or cessation of heartbeat associated with bradycardia and heart failure (Fishbach and Talaska, 2000).

Sodium ion is the principal extracellular cation and its plasma concentration is in equilibrium with that in the interstitial fluid. Therefore, determination of serum sodium ion level is representative of its extracellular fluid concentration (Carreto and Oparil, 2000). It is also directly related to the osmotic pressure and volume of the plasma since water will often follow by diffusion. Consequently, loss of sodium ions leads to dehydration and its retention causes edema and hypertension (Tierney *et al.*, 2001). Therefore, in many hypertensive patients a reduction in sodium intake lowers blood pressure (Nurminen *et al.*, 1998).

Normal serum or plasma sodium ion level is 135-145 mmol/l. Values less than 120 mmol/l (hyponatremia) occur if there is a large loss of gastrointestinal secretions due to diarrhea, severe gastrointestinal disturbances of any sort and diuretic therapy (Tierney *et al.*, 2001). Values greater than 160 mmol/l serum (hypernatremia) occurs in severe dehydration owing to inadequate intake of water or excessive water loss. Serum plasma chloride levels are 98-108 mmol/l and their levels follows sodium (Fishbach and Talaska, 2000).

2.5.2 Hypothermic effects

Plant extracts may possess both antihypertensive and hypothermic activity. According to Szentmiha'lyi, (1998); Okpanyi and Ezeukwu, (1981) and Metowogo *et al.*, (2008) *U. dioica*, *A. indica* and *A. secundiflora* respectively possess hypothermic effect. Hypothermia is a body temperature less than 36°C and is divided into three stages: body temperature between 35-32°C is called mild hypothermia, 32-30°C moderate hypothermia while less than 30°C is

deep hypothermia (Rudlof *et al.*, 2007). Primary hypothermia is low body temperature resulting from environmental changes while secondary hypothermia results from a medical illness or drug effect (Polderman, 2009). Hypothermia can have adverse effects on cardiovascular system such as vasoconstriction at moderate and severe level (Zheng *et al.*, 2001). In addition, intracellular movements of potassium, magnesium and phosphate ions during hypothermia lead to lowered serum concentrations of these ions and may produce serious arrhythmias (Kudenchuk, 1999).

Secondly, hypothermia progressively depresses the CNS and metabolism as the core temperature drops. This decreases tissues' oxygen consumption at lower temperatures causing shivering (Akca, 2005). Thirdly, hypothermia increases a patients' vulnerability to diseases such as pneumonia because of vasoconstriction and impaired immunity. For instance, patients with a core temperature of 1.5°C to 2.0°C below the normal (35.5°C - 35.0°C) have an approximately 19% rate of infection as opposed to a 6% rate of infection in normothermic patients and a 21% mortality rate at body temperatures ranging between 28-32°C (Kudenchuk, 1999). Half of the recorded deaths occur in individuals older than 65 years (Polderman, 2009). It is therefore necessary to investigate the potential hypothermic activity of antihypertensive herbal medicine so that caution may be taken when administering to the patient.

2.6 Extraction of *A. secundiflora, A. indica and U. dioica* leaves

Aloe as an herbal medicine is extracted from aloe juice and therefore does not require use of solvent for extraction. Overheating should be avoided as this produces black powder which is

of poorer qualities (Trease and Evans, 1978). In addition, heat damages important nutrients and medicinal properties (Fairbairn, 1976).

According to Sultana, *et al.*, (2009) plant materials are extracted using either water or an organic solvent to obtain the medicine. The process of extraction for a particular compound or multiple compounds are dependent on the solubility of the rich components in an extraction media such as water, organic solvent or a mixture of both due to property of solubility of the compounds. We can therefore not generalize the extraction of isolated compounds from various herbs (Sultana, *et al.*, 2009).

Since ethanol has both polar and non polar properties it is capable of extracting both organic and ionic compounds. The volatile property (low boiling point 78.5°C) of ethanol makes it easy to recover from the extract compared to water. Moreover, ethanol is relatively less toxic in mammals and does not leave traces of taste nor odor as compared to other solvents such as methanol (ibid). It is therefore preferred as an organic solvent especially in extraction of biologically active compounds in formerly living tissues (Blumenthal *et al.*, 2000). However, when taken in large quantities, it causes an increase in blood pressure and dehydration and therefore the need to recover from the extract (Fuchs *et al.*, 2001). Therefore the study prefered it for the extraction of *A. indica and U. dioica* leaves.

2.7 Formulation of plant extracts into syrup

Pharmaceutical formulation is the process in which different chemical substances are combined to a final medicinal product and is becoming popular as the market for medication continues to grow rapidly. The purpose of formulation is to make drugs or plant extracts palatable, acceptable to people, determine the dosage, preserve and administer with ease. The drug or extract must be combined with inactive additives by a method which ensures that the quantity of the active compound present is consistent in each dosage unit. The dosage should have uniform appearance with an acceptable taste (Sultana *et al.,* 2009).

Initially, simple preparation is developed for use in phase 1 clinical trials. Prove of the long term stability of these formulation is not required as they are to be used/tested in a matter of days. The drug load (the ratio of the active drug/extract to the total content of the dose) should be considered for homogeneity and efficient flow (Nocent *et al.,* 2001). Sweeteners are added to disguise the taste and make the drug/extract palatable and therefore acceptable to the people. Other additives are added to control the effect of the drug or extract by sustaining the absorption rate (Simler *et al.,* 2008).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Research design

The study adopted experimental research design which involves manipulation of independent variables to determine their effect on a dependent variable (Mugenda and Mugenda, 1999). Experimental methods are used to establish causal relationships between variables (ibid). In this study the independent variables were solvents of 60% ethanol and water and, temperature in the extraction process whereas the dependent variable was extract yield in grams of *A. indica* and *U. dioica.* In determining the anti-hypertensive effect; the independent variables were dosage of 10, 20, 40, and 80mg/kg of *A. secundiflora*, cold 60% ethanolic extract of *A. indica* and *U. dioica* leaves formulated syrup and, time in hours. The dependent variable was blood pressure in mmHg.

3.2.1 Extraction of Aloe juice

Fresh Aloe leaves were obtained from Maralal in Samburu County during the dry season. This is because the content of anthraquinones reaches a maximum concentration in the summer and lowest in winter (Trease and Evans, 1978 and Okamura, 1996). Thick aloe leaves were selected because they have more juice. Enough leaves are left to allow the plant continue growing. Aloe juice was obtained by cutting off the outermost leaves transversely from the base and the sap that oozes out was collected in a sliding vat. When the leaves are cut the aloetic juice flows out and therefore no pressure was applied to avoid contaminating the aloe juice with mucilage (Lourdes *et al.*, 2008).

The medicinal values of Aloes are commonly investigated using Aloe juice and gel and not the whole leaf powder. For that reason, this study did not determine the total extract yield of *A. secundiflora* leaf using 60% ethanol and water. The juice was dried into a fine dark brown powder by heating to evaporation at 60^{0}C (Fairbairn, 1976). The dried powder was formulated as described in the formulation section of materials and methods and was designated *A. seccundifora* syrup.

3.2.2 Extraction of *A. indica* and *U. dioica* leaves using cold 60% ethanol and water

Fresh *A. indica* leaves were collected from Kilifi and identified at the herbarium in the School of Biological Sciences University of Nairobi where preserved specimen samples were deposited while the rest of the leaves were dried under a shade (Sofowora, 1993). Fresh *U. dioica* leaves that had not flowered were collected from Molo in Nakuru District. This is because nettle leaves from plants that have already flowered have been reported to affect the kidneys thereafter (Bone and Mill, 2000).

The shoots were cut off just above the root rejecting any infested or insect-beaten leaves. They were tied into bunches of 6 to 10 shoots and these were hung over strings in a well ventilated room. The leaves were dried in an oven at room temperature of 30 ^{o} to a constant weight. Heat was avoided because it may breakdown the links of the chemical components and this would affect their biological functions (Grindlay and Reynolds, 1986).

The dried leaves were pulverized into a fine powder which could pass through 1mm sieve (Mwangi, 1982). Thirty samples (n=30) were used which is a suitable representation in the

experimental study (Mugenda and Mugenda, 1999). As an herbal medicine, *A. indica* and *U. dioica* leaves *are* administered as an aqueous or alcoholic extract. For that reason, the study chose 60% ethanol as an extraction solvent. A concentration of 60% ethanol was chosen because it has a low boiling point than water and therefore takes a shorter time to recover. In addition, ethanol is less toxic than methanol and acetone.

Therefore, 50gm of leaf extract powder was weighed using an E. mettler weighing machine and mixed with 60% ethanol at the ratio of 1:3 w/v. The mixture was left for four days and was constantly stirred using a wooden rod three times a day. After four days the mixture was sieved using a net and the extract filtered. During the second stage of extraction; the residue was soaked for one day, sieved and filtered while in the third stage, the residue was soaked for one hour, sieved and filtered. The filtrate was evaporated at 60^0C using a (Buchi) rotavapor to recover alcohol. It was then freeze-dried to remove water and concentrate it into powder. The powder was stored in a waterproof container to be formulated later. The process was repeated using water as described above.

3.2.3 Extraction of *A. indica* and *U. dioica* using hot solvents of 60% ethanol and water

In hot extraction, 50gm leaf extract powder of *A. indica* and *U. dioica* was soaked in 60% ethanol and water in a hot water bath at 60°C for 24 hours. The mixture was then filtered and evaporated as described in section 3.2.2 above; however, the residue did not go through the second and third stage of extraction.

3.3 Formulation of plant extracts into syrup

Syrup base was prepared by slowly dissolving 2.0% carboxymethyl cellulose (C.M.C.) in cold water that was gently warming while stirring (Sonic stirrer) at 60^0C. Snowflake (5%) was dissolved in cold water and heated in a water bath at 60^oC until thick mixture was obtained. Ten percent of cold 60% ethanolic extract powder of *A. indica and U. dioica* and, 2% of dried *A. secundiflora* juice was dissolved in hot water at 60^0C. Preservatives, 0.1% sodium benzoate and 0.1% citric acid were then added. To make the syrup more palatable, 0.1% sodium saccharin was added as a sweetener while a few drops of organic maroon extract obtained from *Amaranthus* and 0.2% lemon grass oil was added for coloring and as an essence respectively. The extract mixture was added to syrup base and stirred to make uniform syrup. The syrups were labeled as *A. secundiflora, A. indica and U. dioica* syrup.

3.4 Administration of plant extracts

Clinically, oral administration is easier than use of canulla /intravenous route. As a result *A. secundiflora*, *A. indica and U. dioica* syrup was orally given to the male rabbits using a feeding tube and a mouth gag. The syrup was made thinner using normal saline to facilitate smooth flow through the tube. Normal saline was used instead of distilled water to prevent increased osmotic pressure in the blood. The syrup was administered at doses of 10mg, 20mg, 40mg and 80mg /kg. After each experimental period, the rabbits were allowed to recover from the extract effect for two weeks. There were three groups of rabbits; the normotensive (n=6), unilaterally nephrectomised hypertensive (n=6) and renal constricted hypertensive rabbits. Rabbits were the preferred animal model as they are easy to handle during syrup administration and measurement of blood pressure. In addition, male rabbits do

not experience menstrual cycle and therefore would exhibit relatively consistent hormonal level than females during the experimental period (Ganong, 2003).

3.5 Inducing hypertension

3.5.1 Unilateral nepherectomy

The animals were injected intra-muscular with a mixture of 50mg/kg of ketamine and 5mg/kg xylazine. The rabbit was spread on a table with a clean table cloth. A laparectomy was performed by making an incision on the right side to expose the deep fascia and the abdominal muscles using bright light. The right kidney was located and perinephric fat around it removed. Slowly the kidney was pulled out from the body and ligatures were tied using non-absorbable sutures on the right artery until it was occluded. It was then cut as close to the kidney as possible. The place was cleaned using clean cotton wool soaked in normal saline and left for three minutes to observe for any bleeding. The incision was closed in four layers by catgut (absorbable sutures). The wound was sprayed with oxytetracycline. It was covered with sterile gauze and Zinc Oxide tape which was replaced after every three days. After 2 days, the rabbits were given 0.9% (154meq/l) NaCl loading followed by 10% (1700meq/l) NaCl orally until the end of the experimental period. Blood pressure was recorded after two days.

3.5.2. Renal constriction ("Two Kidney" Goldblatt hypertension)

The rabbits were handled as indicated in section 3.5.1 above. However, the right artery was not cut after ligating.

3.6 Measurement of experimental parameters

3.6.1 Blood pressure

Blood pressure was measured from femoral artery on the left thigh of the hind limb. The fur was first shaven using a pair of scissors and then the skin was made clean using a surgical blade 23 g. Digital blood pressure recording machine (MD 300) was used to record the pressure. Blood pressure was measured twice as recommended in WHO MONICA methodology and the mean used in the analysis (The World Health Organization MONICA Project, 1988).

Baseline blood pressure was measured before administering the plant syrup and was used as the control to determine the effect of the extracts on MAP. The rabbits were given *A. secundiflora*, *A. Indica* and *U. Dioica* syrup in the morning daily for 10 days at a dose of 10, 20, 40 and 80mg/kg and blood pressure recorded at the second and sixth hour consecutively. The rabbits were handled by the same individuals throughout the experimental period to prevent erratic changes in blood pressure. In addition, they were wrapped with a towel of the same color and texture when measuring blood pressure to calm them.

3.6.2 Diuretic and laxative effect

The rabbits were orally given 60ml of normal saline and 80mg per kg body weight of the formulated plant extracts. A slanting cage with a hole at one corner was used and urine collected using a funnel and a container. The volume of urine was measured using a measuring cylinder, 12 hours after administration of the plant extracts. Laxative effect was

investigated by observing the texture and frequency of the faeces during the experimental period.

3.6.3 Measurement of serum electrolytes

Blood was collected from a vein in the ear lobe (venipuncture). It was put in BD Vacutainer ® Heparin tube to prevent coagulation since hemolysis causes falsely elevated results for these analytes (Fishbach and Talaska, 2000). The blood was centrifuged at 3000R.P.M for 45 minutes, to separate serum which was used to analyze the level of electrolytes; Na^+, Cl^- and K^+ (Bishop, 2000).

3.6.4 Body temperature

The rabbit was wrapped in a towel and temperature recorded from the anus for 1 minute. The temperature was recorded before and after 2 and 6 hours following administration of the formulated plant extract.

3.7 Statistical analysis

Alternative hypotheses were unidirectional while the data was analysed using Graphpad software. One way ANOVA which is suitable for comparing the means involving one variable at different levels (Mugenda and Mugenda, 1999, Kothari, 1990) was used to: evaluate the mean difference of total extract yield between three stages and one stage extraction process in *A. indica* and *U. dioica* leaves, to compare the mean total extract yield of cold and hot extraction process from the plants and; the hypothermic, diuretic and serum electrolyte effect of the extract syrups. In addition, specific two-group comparisons were

assessed using Bonferroni Multiple Comparison Test while Dunnett's Multiple Comparison Test was used to assess there comparative effect. The antihypertensive effect of *A. secundiflora*, *A. indica* and *U. dioica* syrup on hypertensive rabbits was evaluated using t-test.

CHAPTER FOUR: RESULTS

4.1.1 To compare three stages and one stage extraction process using *A. indica* and *U. dioica* leaves in cold 60% ethanol.

The mean extract yield of the two extraction process was determined using 50g (n=30) samples of dry powder of *A. Indica* and *U. dioica* leaf powder soaked in cold 60% ethanol. Three stages produced high mean total extract yield 7.8g (15.5%) in *A. Indica* and 5.8g (11.6%) in *U. dioica* than one stage process 3.89g (7.8%) and 3.46g (6.92%) respectively (Table 2). The mean extract yield in the second and third extraction stages was significant 2.67g (5.3%) in *A. Indica* and 1.57g (3.14%) in *U. dioica* and 1.25g (2.5%) in *A. Indica* and 0.79g (1.58%) in *U. dioica* respectively. The difference between mean extract yields was significantly high ($df = 5$, $F = 1659$, $p<0.0001$).

	Mean extract yield (g)							
Plant	**Stage 1**	**SE**	**Stage 1**	**SE**	**Stage 1**	**SE**	**Total**	**SE**
U.dioica	3.46	0.0264	1.57	0.03051	0.79	0.02109	5.8	0.1076
A.indica	3.89	0.04377	2.67	0.0287	1.25	0.01486	7.8	0.05901

Table 2 Mean (±SE) extract yield (g) using three stages and one stage extraction process

4.1.2 To compare cold and hot extraction process mean extract yield of *A. indica* and *U.dioica* leaves using 60% ethanol and water

Cold and hot extraction process mean total extract yield was compared using 50g of *A. indica* and *U. dioica* leaf powder (n = 30) in 60% ethanol and water. There was significant mean difference between the mean total extract yields ($df = 7$, $F = 474$, $P < 0.0001$) among the various types of solvents.

Hot 60% ethanolic extraction of *A. indica* leaf produced higher total extract yield 10.6g (21.2%) than cold extraction 7.8g (15.5%). The difference between mean total extract yield was significant ($df = 58$, $t = 15.2$, $p < 0.0001$). Hot water extraction mean total extract yield was higher 8.2g (16.3 %) than cold water extraction 4.8g (9.54%). The difference between mean total extract yield was significant ($df = 58$, $t = 14.2$, $p < 0.0001$). Hot 60% ethanolic extraction produced the highest total extract yield in *A. indica* (Table 3).

Hot 60% ethanolic extraction of *U. dioica* produced higher mean extract yield 6.75g (13.5%) than cold 60% ethanol, 5.8g,(11.6%). The difference between the mean total extract yield was significant ($df = 58$, $t = 6.6$, $p < 0.0001$). Hot water extraction produced higher total extract yield, 15.1g (30.2%) compared to cold water 6.4g (12.8%). The difference between the mean total extract yield was significant ($df = 58$, $t = 37.6$, $p < 0.0001$). Hot water extraction produced the highest mean total extract yield in *U. dioica* (Table 3).

	Mean total extract yield (g) ± SE			
	60% ethanol		water	
Plant extract	**Cold**	**Hot**	**Cold**	**Hot**
A. indica	7.8 ± 0.0979	10.6 ± 0.212	4.8 ± 0.2287	8.2 ± 0.1235
U. dioica	5.8 ± 0.0865	6.8 ± 0.0392	6.4 ± 0.1136	15.1 ± 0.261

Table 3 Mean (±SE) extract yield (g) using cold or hot extraction

4.2 Normal blood pressure of caged male New Zealand white rabbits

The normal mean blood pressure for male New Zealand white rabbits weighing between 2.5-3.00kg ranged between 63.2-98mmHg (MAP). Erratic increases in blood pressure up to 179.8mmHg were observed in cases of change of staff or when the animals were handled unwrapped.

4.3 Hypotensive effect of the plant extracts syrup

4.3.1 Hypotensive effect of *A. secundiflora* syrup on normotensive male rabbits

The hypotensive effect of *A. secundiflora* was immediate, persistent and dose-dependent. However, by the end of the first week of administration of 80mg/Kg, significant hypotension and excessive diarrhea was observed. Moreover, administration of 80mg/kg of *A. secundiflora* for more than 10 days caused extreme loss of weight, diarrhea and dehydration. A dose of 10, 20, 40, and 80mg/kg of *A. secundiflora* syrup on normotensive male rabbits caused a decrease in MAP by 3.7mmHg (4.1%), 9.4 mmHg (10.4%), 14.84 mmHg (17.5%) and 24.3mmHg (27.7%) respectively by the second hour while by the sixth hour MAP

decreased by; 8.0mmHg (8.8%), 16.02mmHg (17.6%), 23.49mmHg (27.7%) and 29.67mmHg (33.8%) respectively. The F value was not significant at 10mg/Kg ($F = 1.237$, $df = 2$, $P = 0.3182$), but was highly significant at 20mg/kg ($F = 10.18$, $df = 2$, $P = 0.0016$), 40mg/kg ($F = 64.89$, $df = 2$, $P < 0.0001$) and 80mg/kg ($F = 39.3$, $df = 2$, $P < 0.0001$).

4.3.2 Hypotensive effect of *A. indica* syrup on normotensive male rabbits

The hypotensive effect of *A. indica* syrup was immediate, persistent and dose-dependent. After the second hour following administration of 10, 20, 40, and 80 mg/Kg MAP decreased by 4.0mmHg (4.7%), 6.4mmHg (7.7%), 12.2mmHg (13.9%) and 22.5mmHg (27.7%) respectively; while after 6 hours MAP decreased by 2.0mmHg (2.3%), 2.9 mmHg (3.5%), 13.8mmHg (15.1%) and 26.8 mmHg (30.5%) respectively. F value was not significant at 10mg/kg ($F = 2.55$, $df = 2$, $P = 0.1114$) but was highly significant at 20mg/kg ($F = 6.385$, $df = 2$, $P = 0.0099$), 40mg ($F = 33.85$, $df = 2$, $P < 0.0001$) and 80mg ($F = 127.7$, $df = 2$, $P < 0.0001$).

4.3.3 Hypotensive effect of *U. dioica* syrup on normotensive male rabbits

The hypotensive effect of *U. dioica* syrup was immediate, dose-dependent but was persistent only at higher dosage of 80mg/kg. By the second hour after administration of 10, 20, 40, and 80 mg/Kg MAP decreased by 8.9mmHg (11.0%), 11.5mmHg (13.6%), 15.1mmHg (18.3%) and 20.6mmHg (24.72%) respectively while after the sixth hour it decreased by 1.54mmHg (1.9%), 1.9mmHg (2.3%), 5.8mmHg (6.7%) and 21.62mmHg (25.94%) respectively.

F value was highly significant at all levels of dosage: 10mg/kg ($F = 49.9$, $df = 2$, $P < 0.0001$),

20mg/kg (F = 41.04, df = 2, P < 0.0001), 40mg (F = 52.48, df = 2, P < 0.0001) and 80mg (F =178.9, df =2, P<0.0001).

Dosage in mg/kg	Time in hours	*A. secundiflora* syrup		*A. indica* syrup		*U. dioica* syrup	
		q value	95% CI of diff	q value	95% CI of diff	q value	95% CI of diff
10	2	0.7254^{ns}	-8.757 to 16.17	2.258^{ns}	-0.3214 to 8.335	9.349***	6.616 to 11.29
	6	1.572^{ns}	-4.434 to 20.49	1.131^{ns}	-2.321 to 6.335	1.612^{ns}	-0.7922 to 3.879
20	2	2.644*	0.7312 to 18.14	3.57**	2.040 to 10.84	8.309***	10.68 to 19.56
	6	4.489***	7.315 to 24.73	1.65^{ns}	-1.424 to 7.377	1.025^{ns}	-2.568 to 6.308
40	2	7.118***	9.758 to 19.93	6.649***	7.714 to 16.65	10.24***	8.794 to 14.29
	6	11.26***	18.40 to 28.57	7.521***	9.311 to 18.25	5.154***	3.059 to 8.555
80	2	24.3***	15.60 to 33.00	8.432***	15.99 to 29.01	15.97***	17.46 to 23.75
	6	29.67***	20.97 to 38.37	15.97***	36.11 to 49.13	16.75***	18.48 to 24.77

Table 4 Comparative hypotensive effect of *A. secundiflora*, *A. indica* and *U. dioica* syrup in nomortensive male rabbits

4.3.4 Comparative hypotensive effect of *A. secundiflora*, *A. indica* and *U. dioica* syrup on normotensive male rabbits

The hypotensive effect of the three plant extracts was compared at different amount of dosage against time using Dunnet's Multipple Comparison Test (Table 4). All the plant syrups (*A. secundiflora*, *A. indica* and *U. dioica)* showed immediate dose-dependent hypotensive activity. There was no significant hypotensive activity at 10mg/kg in both *A. secundiflora* and *A. indica* while similar dose of *U. dioica* showed significant decrease in MAP by the second hour. At 20mg/kg, *A. secundiflora* showed significant decrease in MAP at all levels of time, while the hypotensive activity of *A. indica* and *U. dioica* was not persistent to the sixth hour. At 40 and 80mg/kg, all the plant extracts showed significantly high hypotensive activity both after the second and sixth hour ($P < 0.0001$).

4.3.5 Comparative anti-hypertensive effect of *A. secundiflora*, *A. indica* and *U. dioica* on hypertensive male rabbits

In rabbits with unilateral nephrectomy salt loaded with 10% NaCl orally administered for eight weeks, MAP increased from 72mmHg to121mmHg (40.1%). However, after administration of 80mg/Kg of formulated *A. secundiflora, A. indica and U. dioica* syrup for 10 days, MAP decreased by 31.6 ± 9.98mmHg (25.9%); 18.8 ± 7.11mmHg (15.4%) and 14.72 ± 6.08mmHg (12.1%) respectively *A. secundiflora, A. indica and U. dioica* syrup.
The decrease in MAP after administration of each extract was tested for significance using ANOVA, the t value at the subscribed P value of 0.05 was more for aloe than stinging nettle and neem (Table 5).

In rabbits with unilateral renal constriction and on 10% NaCl loading given orally for 8 weeks, MAP increased significantly from 71mmHg to 136mmHg (61%). After administration of 80mg/Kg of formulated *A. secundiflora, A. indica and U. dioica* syrup;

MAP decreased by 53.5 ± 7.51mmHg (39.5%), 30.7 ± 8.20mmHg (22.7%) and 40.0 ± 9.88mmHg (29.6%) respectively. Using ANOVA; the means were significantly different, the t value at the subscribed p value of 0.05 was more for *A. secundiflora* than *U.dioica* and *A. indica* (Table 6).

Plant leaf extract syrup	**P value(p<0.05)**	**t value**	**d.f**	**Change in MAP ±SE**
A. secundiflora	0.005	3.16	10	31.6 ± 9.98
A. indica	0.039	2.37	10	14.72 ± 6.08
U. dioica	0.012	2.98	10	18.8 ± 7.11

Table 5 Comparative anti-hypertensive effect of 80mg/kg of *A. secundiflora, A .indica and U. dioica* syrup on unilaterally nephrectomized hypertensive male rabbits

Plant leaf extract syrup	**P value(p<0.05)**	**t value**	**d.f**	**Change in MAP ±SE**
A. secundiflora	0.0001	7.13	10	53.50 ± 7.509
A. indica	0.0028	3.74	10	30.70 ± 8.203
U. dioica	0.0019	4.05	10	40.00 ± 9.888

Table 6 Comparative antihypertensive effect of 80mg/kg of *A. secundiflora A. indica* and *U. dioica* syrup on right renal constricted hypertensive male rabbits

4.4 Comparative diuretic effect of *A. secundiflora*, *A. indica* and *U. dioica* syrup on male rabbits

Mean urine volume produced by the rabbits administered with 80mg/kg of each plant leaf extract was compared with the placebo. *U. dioica* demonstrated the highest diuretic effect comparable to Furosemide. It was followed by *A. secundiflora* while *A. indica* demonstrated the lowest effect (figure 4). However, *A. secundiflora* syrup effect occurred 10 hours subsequent to administration.

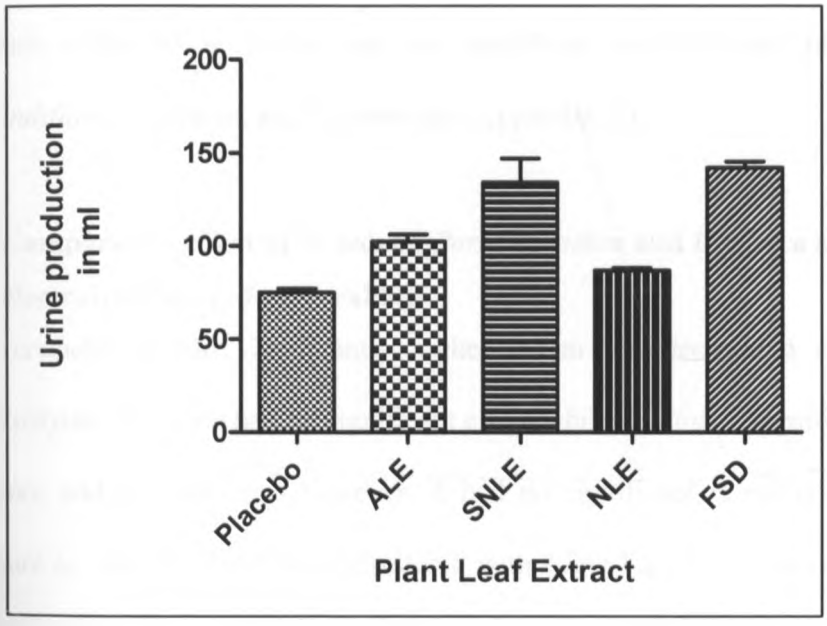

Figure 4 Comparative diuretic effect *A. secundiflora*, *A. indica* and *U. dioica* syrup on male rabbits

KEY

ALE- *A. secundiflora* syrup

NLE- *A. indica* syrup

SNLE- *U. dioica* syrup

FSD-Furosemide

A. secundiflora, *A. indica*, *U. dioica* syrups and Furosemide increased mean urine volume by 27.5ml (36.7%), 11.83ml (13.6%), 59.33ml (44.9%) and 67ml (47.2%) respectively (Fig. 4). Rabbits that received *U. dioica* syrup recorded the highest volume of urine (165ml) than Furosemide (155) during the experimental period even though the mean was lower. The mean urine volume effect of *A. secundiflora*, *A. indica*, *U. dioica* and Furosemide was significantly different ($F = 21.73$, $df = 3$, $p < 0.0001$). The comparative significance of the mean urine volume increase was tested using Dunnett's Multiple Comparison Test. The diuretic effect of *A. indica* was not significant while it was highly significant in *A. secundiflora*, *U. dioica*, and Furosemide (Appendix A).

4.5 Comparative effect of *A. secundiflora*, *A. indica* and *U. dioica* syrup on serum electrolytes level of male rabbits

A. secundiflora had significantly higher serum ion decreasing effect on all the three electrolytes, *A. indica* had no significant effect while *U. dioica* significantly decreased serum sodium and chloride ions however, it had no significant effect on serum potassium ions (Figure 6, 7and 8). Rabbits administered with 80mg/Kg of *A. secundiflora*, *U. dioica* and *A. indica* syrup decreased serum sodium ion levels by 6.8mmol/l (4.99%), 5.85mmol/l (4.3%) and 0.933mmol/l (0.68%) respectively. The mean change in serum sodium level was significantly different ($F = 12.9$, $df = 3$, $P < 0.0001$).

Serum potassium ion level decreased by 1.028mmol/l (21.04%), 0.032mmol/l (0.65%) and 0.062mmol/l (1.26%) in *A. secundiflora*, *U. dioica* and *A. indica* syrup respectively. The

mean change in serum potassium level was significantly different ($F = 23.22$, $df = 3$, $P < 0.0001$).

Serum chloride ion levels decreased by 10.45mmol/l (9.74%), 7.07mmol/l (6.6%) and 2.35mmol/l (2.7%) in; *A. secundiflora*, *U. dioica* and *A. indica* syrup respectively. The mean change in serum sodium ion level was significantly different ($F = 40.64$, $df = 3$, $P < 0.0001$). The comparative effect of the three plant extracts on mean change in serum ion level was tested using Dunnett's Multiple Comparison Test (Appendix B).

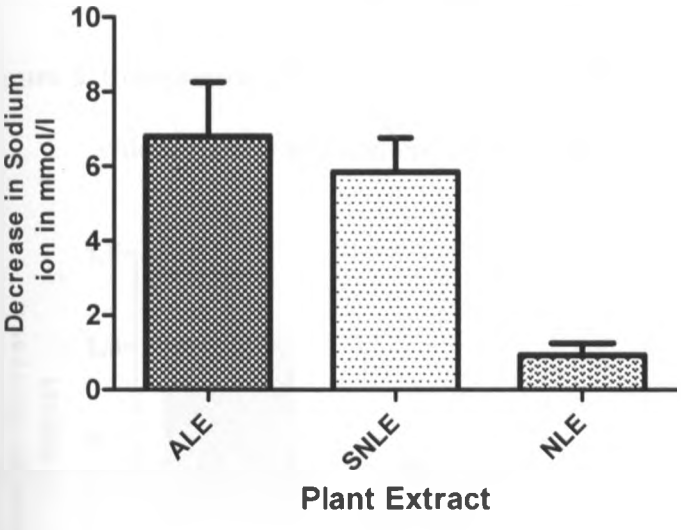

Figure 5 Comparative effect of *A. secundiflora*, *U. dioica* and *A. indica* syrup on serum sodium ion concentration in male rabbits

KEY (Fig. 5, 6 and 7 above)

ALE- *A. secundiflora* syrup SNLE- *U. dioica* syrup

NLE- *A. indica* syrup

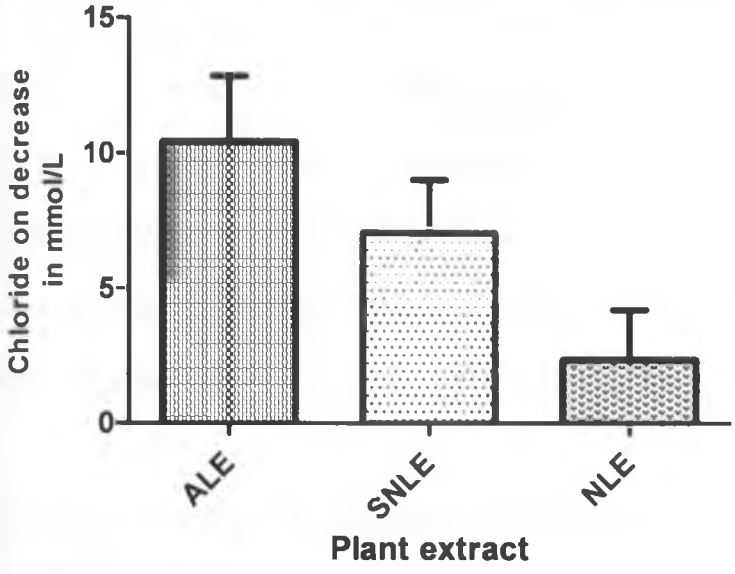

Figure 3 Comparative effect of *A. secundiflora*, *U. dioica* and *A. indica* syrup on serum chloride ion concentration in male rabbits

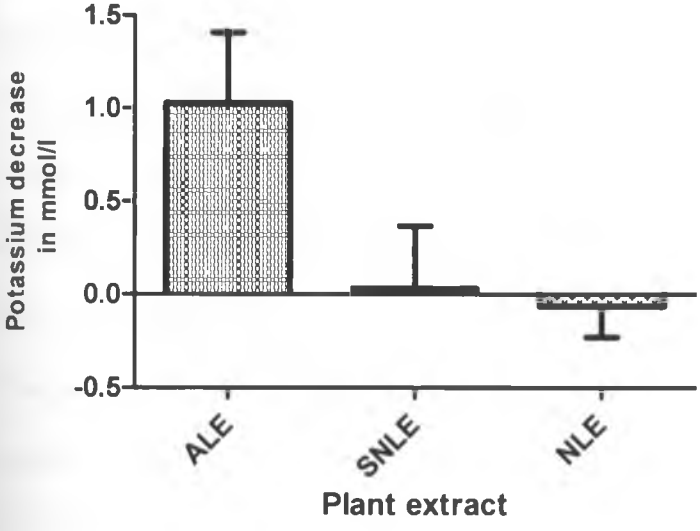

Figure 4 Comparative effect of *A. secundiflora*, *U. dioica* and *A. indica* on serum potassium ion concentration in male rabbits

4.6 Comparative laxative effect of *A. secundiflora*, *A. indica* and *U. dioica* syrup in male rabbits

There was no observed laxative effect in rabbits after administration of 80mg/kg of *A. indica* and *U. dioica* syrup. The study observed frequent dose-related production of loose stool from the second day after administering doses of 10, 20, 40 and 80mg/kg of *A. secundiflora.* Adose of 80mg/kg showed excessive diarrhea by the 7th day furthermore, the rabbits were weak, dehydrated and had lost body weight significantly by the 10th day.

4.7 Comparative hypothermic effect of *A. secundiflora*, *A. indica* and *U. dioica* syrup on male rabbits

Normal body temperature of male New Zealand White rabbits varied greatly between individual rabbits, ranging between 38^{0}C and 40^{0}C. Consequently, body temperature recorded before administration of the extracts was used as the baseline and used to determine any decrease in each rabbit after the second and sixth hours respectively. After administration of 80mg/kg of formulated *A. secundiflora*, *A. indica* and *U. dioica* syrup, temperature decreased by 0.4°C (1%), 0.4°C (1%) and 0.3°C (0.8%) respectively after the second hour while by the sixth hour the temperature decreased by 0.5 (1.26%), 1°C (2.6%) and 0.4°C (1%) respectively.

F value was not significant for *U. dioica* (F = 1.973, df = 2, P = 1.735) whereas it was significant for *A. indica* (F = 1.992, df = 2, P = 0.034) and *A. secundiflora* (F = 4.900, df = 2, P = 0.023). The significance of the mean decrease in temperature at different level of time in all the syrups was comparatively tested using Dunnett's Multiple Comparison Test (Appendix C)

CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1: Discussion

5.1.1 Comparative total extract yield of *A. indica* and *U. dioica* leaf in a three stages extraction process using cold and hot 60% ethanol and, water.

The findings from this study showed that extracting *A. indica* and *U. dioica* leaves using a three stage process in cold 60% ethanol produces significantly higher total extract yield than one stage process. This was as a result of the additional extract yield from the second and third extraction stages. In addition, hot extraction yielded significantly higher than cold extraction. This suggests that, combining use of hot solvent and three stages extraction process is more efficient as it yields maximum extract within a short time. With these novel findings, it is possible to estimate the minimum amount of herbal materials required to yield the needed extracts. This will prevent wasteful harvesting of medicinal plants and henceforth promote conservation of the environment. The findings also show that use of hot 60% ethanol and water to extract *A. indica* and *U. dioica* leaves respectively is the most efficient. Moreover, they also indicate that most of the chemical components in *A. indica and U. dioica* leaves are non polar and polar respectively (Sultana, *et al.*, 2009).

5.1.2.1 Comparative hypotensive effect of *A. secundiflora*, *A. indica* and *U. dioica* syrup on normotensive rabbits

The effective hypotensive dosage of *A. secundiflora*, *A. indica* and *U. dioica* syrup and their potential side effects have not been consistently reported in various reviews but in this study, the hypotensive potency of the extracts at various dosage level and other potential side effects were comparatively determined. To the best of my knowledge such a comparative

study has not been done. The hypotensive activity of *A. secundiflora* could have been caused by aloe-emodin which has been reported to cause 26%, 52% and 79% fall in MAP at the corresponding doses of 0.5,1 and 3mg/kg in rats (Saleem *et al.*, 2005). According to Atherton, (1997b) this is due to the transit time taken by aloin to the colon where it is converted to active metabolite (aloe-emodin-9-anthrone). This may also have caused persistent and significantly high hypotensive activity observed by the sixth hour (Table 4).

The immediate hypotensive effect of *A. secundiflora* syrup observed by the second hour may have been caused by vasodilation of capillaries as reported by Reynolds and Dweck, (1999) consequently decreasing MAP. According to Waihenya *et al.*, (2002) Aloe juice contains acemannan which stimulate production of nitric oxide (NO) causing vasodilation. In addition the increased diuretic effect of *A. secundiflora* could also have significantly reduced the extracelluar fluid/blood volume resulting in significant further decrease in MAP observed by the sixth hour (Appendix A).

The hypotensive effect of 60% ethanolic *A. indica* syrup demonstrated immediate and persistent dose-related activity that lasted for more than six hours at doses of 40 and 80mg/kg given orally. Similarly, Koley and Lal, (1994) reported immediate, sharp and persisted dose-related fall in blood pressure in cats administered *A. indica* leaf extract. The same Koley and Lal, (1994) adds that, alcoholic leaf extract of *A. indica* administered intravenously produced similar results. Moreover, intravenous aqueous extract produced comparable effect in anesthetized dogs (Willcox *et al.*, 2004).

The study showed that the hypotensive activity of 60% ethanolic extract of *A. indica* syrup at lower doses of 10 and 20mg/kg administered orally was not significant as it did not persist to the sixth hour. This observation contradicts findings of Thompson and Anderson, (1978) who observed that intravenous doses of 5-200mg/kg leaf extract induced profound dose dependent hypotension in rabbits. This suggests that different route of administration may produce different effect.

The hypotensive effect was caused by nimbidin present in *A. indica* leaves (Subapriya and Nagini, 2005). According to Singh *et al.*, (1987) nimbidin and most other limonoids interfere with the CNS neurotransmission as they are sufficiently lipophillic to cross the blood-brain barrier. Since the activity of atropine failed to prevent the hypotensive activity of *A. indica* leaf extract, Thompson and Anderson, (1978) suggested an inhibitory effect on adrenergic receptors or the sympathetic nervous system hence decreased MAP. Furthermore, Koley and Lal, (1994) reported that antihistaminic properties (H_2- receptor) of nimbidin and xanthine demonstrated vasodilatatory effect; these may have caused further decrease in MAP as observed in this study.

From the findings in this study, 60% ethanolic *U. dioica* syrup demonstrated immediate and significant dose-related hypotensive activity at 40 and 80mm/kg. These findings concur with previous studies which observed marked hypotension and bradycardia at a dose of 26.6mg/kg of aqueous extract given by canulla to cats (Fairbairn, 1976). Similarly, Adamski and Bieganska, (1984) observed that 25mg/kg of nettle herb produced rapid decrease of 31.7% on

blood pressure of rats. The immediate hypotensive effect is possibly due to significant diuretic effect observed in this study. This observation concurs with the findings of Tahri *et al.*, (2000) and Szentmiha'lyi, (1998) who reported that *U. dioica* preparation given for two weeks produced slight decrease in systolic blood pressure in patients with cardiac or chronic venous insufficiency accompanied by diuretic and natriuretic effect.

The persistent hypotensive effect *U.dioica* may have been due to its vasodilatory and bradycardia activity (Chubrasik *et al.*, 2009). Since atropine showed no effect on *U. dioica* bradycardia activity, a mode of action in the CNS via alpha-adrenoreceptors has been suggested (Legssyer *et al;* 2002). Further vasodilatory activity of *U. dioica* may have resulted from the effect of xanthophylls and high content of potassium ions present in *U. dioica* (Chubrasik *et al.*, 2009). However, the response at 10 and 20mg/kg was not significantly persistent up to the sixth hour. Similarly, Szentmiha'lyi, (1998) observed that perfusion of aqueous extract from nettle herb reduced arterial blood pressure by 15% at lower dose of 4mg/kg/hr but the effect was reversible in about 1 hour of recovery. However, at a higher dose of 24mg/kg/hr the extract persistently decreased blood pressure by 38%.

The hypotensive effect of *A. secundiflora, A. indica* and *U. dioica* syrup was compared in this study to determine which possessed the highest activity. Findings in this study demonstrated that *A. secundiflora* had the highest hypotensive effect at a lower dose due to its highly significant diuretic and laxative effect. This was comparable to highly significant diuretic effect of *U. dioica* syrup that caused significant hypotensive effect than *A. indica*

syrup. The vasodilatory and diuretic effects of flavonoids and high potassium content 448:1 K^+: Na^+ ratio in *U. dioica* may have further decreased MAP (Legssyer *et al.,* 2002; Tahri *et al.,* 2000 and Szentmiha'lyi, 1998). *A. indica* demonstrated the lowest hypotensive effect compared to *A. secundiflora* and *U. dioica* syrup as it did not demonstrate significant diuretic activity and laxative effect. However, the effect was persistent than *U. dioica* may be as a result of nimbidin which has vasodilatory, anti-histaminic and CNS suppressing effect (Pillai and Santhakumari, 1984).

According to Atherton, (1997a) the smallest dosage to be used in treating a disease should be the lowest significant amount to achieve effectiveness with minimum toxic effect within the shortest time. The study observed that the minimum significant hypotensive dosage for six hours was 20mg/kg in *A. secundiflora* and 40mg/kg in *A. indica* and *U. indica* (Table 4.3). These findings provide the minimum effective hypotensive dosage consequently prevent under-dosage and more importantly over-dosage in future as this is prevalent in herbal medicine prescriptions.

5.1.2.2 Comparative anti-hypertensive effect of *A. secundiflora*, *A. indica* and *U. dioica* syrup on unilateral nephrectomized and renal constricted hypertensive male rabbits

This study showed that *A. secundiflora*, *A. indica* and *U. dioica* syrup had higher antihypertensive effect on renal constricted induced hypertension (Two-kidney, Goldblatt hypertension) than in nephrectomized male rabbits because the former is associated with higher extracellular fluid volume than the latter. In addition to 10% salt loading, the

constriction caused the ischemic kidney to retain salt and water because of decreased renal arterial pressure while the normal opposite kidney retained salt and water because of rennin produced by the ischemic kidney. However, in nephrectomized rabbits, fluid and salt retention was caused by 10% salt loading only (Guyton and Hall, 2000).

This study observed increased diuretic and laxative effects on rabbits administered with *A. secundiflora* syrup (Atherton, 1997b; Odes and Madar, 1991). As a result, it significantly reduced MAP by decreasing extracellular fluid volume (Ganong, 2003) and this suggests why it demonstrated the highest anti-hypertensive activity. Due to its diuretic effect (Appendix A) *U. dioica* syrup demonstrated higher antihypertensive effect than *A. indica.* Diuretic effect is associated with decreased extracellular fluid volume as reported by Tahri *et al.*, (2000).

5.1.3 Comparative diuretic effect of 80mg/kg *A. secundiflora*, *A. indica* and *U. dioica* syrup on male rabbits

A. secundiflora syrup produced delayed but highly significant diuretic effect as an activity of aloin in the colon. These findings corroborates with Atherton, (1997b) observation that Aloe causes delayed diuretic effect as a result of the transit time taken by aloin from the small intestine to the colon. Aloins, aloinosides and hydroxyaloins contained in Aloe juice are converted to aloe-emodin-9-anthrone through enzymatic or bacterial reductive cleavage activity in the large intestines/colon. Aloe-emodin-9-anthrone increases the leakiness of the tight junctions, causes stimulation of active secretion of fluids/water and electrolytes into the

lumen (secretagogue effect) and inhibits rearbsorption of fluids from the colonic epithelial cells (antiarbsorptive effect).

Findings from this study indicated that the diuretic effect of *A. indica* (13.6 %) was not significant and did not demonstrate an obvious trend. This observation concurs with Van der Nat *et al.,* (1991) observation that *A. indica* causes decreased or no-urine production in rabbits. However, they contradict the findings of Koley and Lal, (1994) who observed that *A. indica* leaf extract sodium nimbidinate caused diuretic effect. The observation of Koley and Lal can not be valid because *A. indica* leaf extract has been observed in this study (Appendix B) to have insignificant decreasing effect on serum electrolytes which is associated with diuresis. Moreover, Ogbuewu *et al.,* (2001) observed that *A. indica* leaf extract possess anti-diuretic effect.

U. dioica showed the highest significant diuretic effect that is closely similar to Furosemide. The diuretic property of *U. dioica* was first reported by Greek physicians Dioscorides and Galen in the first century. Similarly Tahri *et al.,* (2000) observed that *U. dioica* administered to individuals with cardiac or chronic venous insufficiency demonstrated diuretic effect. According to Tita *et al.,* (1993) the diuretic effect is due to its flavonoids and high potassium content 448:1 K^+: Na^+ ratio.

Therefore, this study suggests that minimum hypotensive dosage of the *A. secundiflora* and *U. dioica* can be administered to individuals with hypertension caused by increased

extracellular volume. However, concurrent internal use with loop diuretics such as Furosemide, thiazides and potassium wasting drugs should be avoided because it would enhance their effect. This supports Langmead *et al.,* (2004) view that individuals should be informed that oral use of Aloe can cause severe serious hypokalemia, dehydration and electrolyte imbalance and therefore would not be recommended.

5.1.4 Comparative effect of 80mg/kg *A. secundiflora*, *A. indica* and *U. dioica* syrup on serum electrolyte level of male rabbits

According to the findings of this study *A. secundiflora* demonstrates the highest significant effect in decreasing serum electrolytes. According to Atherton, (1997b) this could have been caused by aloin active metabolite (aloin-9-anthrone) in the colon which causes secretagogue and non-absorptive effects (diuretic effect) and, increased peristalsis (laxative effect). Consequently, this results to excessive loss of sodium ions (natriuresis) (Lourdes *et al.,* 2008) and potassium ions (hypokalemia) by (21.04 %) (Appendix B). The combined effect of diuretic and laxative effect caused significant hypotension observed in this study (Table 4). As a result of these effects, the study suggests the lowest significant hypotensive dosage of *A. secundiflora* and *U. dioica* as 20mg/kg and 40mg/kgrespectively. Consequently, this will prevent serious toxic effect such as paralysis of the intestinal muscles, hyperaldosteronism and arrhythmia (Bolkent *et al.,* 2004).

From this study, *A. indica* syrup showed no significant decreasing effect on serum electrolyte. Similarly, Ogbuewu *et al.,* (2001) reported that *A. indica* leaf extract possess anti-diuretic effect whereas 10ml/day of *A.indica* oil administered to rabbits stimulated active

rearbsorption of sodium electrolyte ions from the gut. The same Ogbuewu *et al.*, adds that *A. indica* leaves added to rabbit feed improved the uptake of serum sodium and chloride from the kidney. These suggested that *A. indica* leaves could be having some chemical component that increases level of serum electrolyte such as potassium as observed in this study (Fig 7).

The study observed that *U. dioica* syrup caused significant decrease in serum sodium and chloride ions. On the same vein, Szentmiha'lyi, (1998) observed that perfusion of dry aqueous extract from nettle herb at a dose of 4mg/kg/hr and 24mg/kg/hr caused diuretic and natriuretic effects. This report implys that the diuretic activity of *U. dioica* syrup demonstrated in this study caused loss of ions through urine. However, there was no significant decrease of potassium ions because *U. dioica* has very high potassium content (Tita *et al.*, 1993).

Consequently, as previously suggested in this study, *U. dioica* and *A. secundiflora* syrup should not be used concurrently with potential diuretics and ACE inhibitors as they may potentiate their effect causing hypotension, dehydration, natriuresis and hypokalemia. Moreover, *U. dioica* should be carefully used as a vegetable especially in individuals with diarrhea or decreased serum electrolyte.

5.1.5 Comparative laxative effect of 80mg/kg *A. secundiflora*, *A. indica* and *U. dioica* syrup in male rabbits

From the observation made in this study, *A. secundiflora* demonstrated significant laxative effect in rabbits across all levels of dosage on the second day after administration of the

extract. This observation concurs with previous findings of Odes and Madar, (1991) who reported that Aloe increased the frequency of stool evacuation from 1.1 ± 0.2 evacuations/ day whereas transit time decreased from 64 ± 20 hours to 18 ± 4 hours. In addition, 16 out of 19 chronically constipated adults reported improvement ($P<0.05$). According to Atherton, (1997b) aloe-emodin causes irritation of the colonic mucosa which in turn precipitates active secretion of mucous that stimulates peristalsis. The result is increased propulsion and reduced transit time (laxative effect). The effect was observed after 10 hours because of the transit time of the aloin to the colon where it is metabolized into active compound (ibid). World Health Organization and European Medicines Agency recommends that the dosage of Aloe as a laxative should not exceed 50mg/day. In addition, few studies support the use of Aloe juice for internal use (Ishii *et al.*, 1998).

On the same vein, this study suggests that the laxative and diuretic effect of *A. secundiflora* syrup may have significantly caused higher hypotensive activity than the other extracts (Table 4). Consequently, it recommends 20mg/kg of *A. secundiflora* as the lowest dosage for not more than 10 days while the maximum dosage should not exceed 80mg/kg. *A. indica* and *U. dioica* did not demonstrate any observable laxative effect and therefore may not cause severe hypokalemia or dehydration.

5.1.6 Comparative hypothermic effect of 80mg/kg of *A. secundiflora*, *A. indica* and *U. dioica* syrup in male rabbits

From the study, *A. secundiflora* demonstrated significant hypothermic effect. This observation concurs with findings of Khattak *et al.*, (1985) who reported that Aloe

significantly demonstrated hypothermic effect in hyperthermic rats induced by yeast injection.

A. indica showed significant hypothermic effect. These results are in agreement with the findings of Singh *et al.,* (1987) who reported that, ethanolic extract of *A. indica* leaf showed a dose dependent hypothermic effect. According to Metowogo *et al.,* (2008), the effect is caused by nimdidin which causes mild suppressive effect on the central nervous system.

This study did not find significant hypothermic effect of *U. dioica* syrup. This contradicts findings of Baraibar *et al*., (1983) and Tahri *et al.,* 2000 who reported that aqueous extract of *U. dioica* lowered the body temperature of rats. This suggests that the rabbit respondent differently compared to the other animal models previously used or 60% ethanolic extracted none or insignificant hypothermic chemical components. Therefore, the findings suggest that over-dosage with *A. secundiflora* and *A. indica* should be avoided as it may result in serious physiological effects associated with hypothermia such as vasoconstriction, hypokalemia, arrhythmia, decreased metabolism and lowered immune system (Kudenchuk 1999, Akca, 2005).

5.2 Conclusion

The study established that;

i. Extraction using cold 60% ethanol produced significantly high total extract yield both in *A. indica* and *U. dioica* leaf extraction process than one stage process.

ii. Hot extraction produced significantly higher total extract yield compared to cold extraction in both *A. indica* and *U. dioica* leaves.

iii. The most efficient extraction solvent of extracting *A. indica* and *U.dioica* leaves is hot 60% ethanol and hot water respectively.

iv. The minimum significant anti-hypertensive dosage for the syrups is 20mg/kg in *A. secundiflora* and 40mg/kg for both *U. dioica* and *A. indica*.

v. *A. secundiflora*, *U. dioica* and *A. indica* possess significant antihypertensive effect in salt loaded unilateral nephrectomised and renal constricted hypertensive male rabbits.

vi. *A. secundiflora* and *U. dioica* showed significant diuretic effect while in *A. indica* it was insignificant.

vii. *A. secundiflora* and *U. dioica* syrup significantly decreased serum electrolytes level while *A. indica* was insignificant.

viii. *A. secundiflora* and *A. indica* syrup showed significant hypothermic effect while *U. dioica* was insignificant.

5.3 Recommendations

i. The antihypertensive effect of the plant extracts should be carried out in clinical trials in collaboration with medical research institutes such as KEMRI.

ii. Other toxic effects of *A. secundiflora*, *U. dioica* and *A. indica* on major body organs should be investigated.

iii. The ministry of health and other stakeholders should embark on intensive public awareness on health risks associated with concurrent use of plant extracts and conventional drug.

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APPENDICES

Appendix A: Comparative diuretic effect of 80 mg/kg *A. secundiflora*, *A. indica* and *U. dioica* syrup on male rabbits in male rabbit

80mg/kg of plant extract	q value	95% CI of difference
A. secundiflora	3.102	-50.62 to -4.375
A. indica	1.335	-34.96 to 11.29
U. dioica	6.692	-82.46 to -36.21
Furosemide	7.557	-90.12 to -43.88

Appendix B: Comparative effect of 80 mg/kg *A. secundiflora*, *A. indica* and *U. dioica* syrup on male rabbits on serum electrolytes in male rabbits

	Sodium ions		Potassium ions		Chloride ions	
80mg/kg of plant extract	**q value**	**95% CI of difference**	**q value**	**95% CI of difference**	**q value**	**95% CI of difference**
A. secundiflora	5.042***	3.374 to 10.23	6.731***	0.6402 to 1.416	10.04***	7.807 to 13.09
A. indica	0.6921^{ns}	-2.493 to 4.359	0.4036^{ns}	-0.4498 to 0.3265	2.259^{ns}	-0.2931 to 4.993
U. dioica	4.338***	2.424 to 9.276	0.2073^{ns}	-0.3565 to 0.4198	6.792***	4.424 to 9.710

Appendix C: Comparative hypothermic effect of 80 mg/kg *A. seccuniflora*, *A. indica* and *U. dioica* syrups on serum electrolytes in male rabbits

	A. secundiflora		*A. indica*		*U. dioica*	
Time in hours	**q value**	**95% CI of difference**	**q value**	**95% CI of difference**	**q value**	**95% CI of difference**
2	2.367^{ns}	-0.01220 to 0.8122	1.474^{ns}	-0.3274 to 1.327	1.482^{ns}	-0.1775 to 0.7275
6	2.959*	0.08780 to 0.9122	2.924*	0.1643 to 1.819	1.887^{ns}	-0.1025 to 0.8025