HYPOTENSIVE ACTIVITY OF 70% ETHANOL EXTRACT OF AERIAL PART OF *RUTA CHALPENSIS BOTH* ***in vivo*** *AND* ***ex vivo*** AND ITS ANTIOXIDANT ACTIVITY

A Thesis Submitted to the School of graduate studies of Addis Ababa University School of Medicine, Collage of Health Science in partial Fulfillment of the Requirements for the degree of Master of Science in Medical Biochemistry.

Frehiwot Teka

November, 2015

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ADDIS ABABA UNIVERISTY

SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared by **Frehiwot Teka Assamo** entiteled : ***"Hypotensive activity of 70% ethanol extract of aerial part of Ruta chalpensis both in vivo and ex vivo and its antioxidant activity"*** and submitted in partial Fulfillment of the Requirements for the degree of Master of Science in Medical Biochemistry complies with regulations of the university and meets accepted standard with respect to originality and quality.

Signed by the examining committee:

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Chair of department of graduate program coordinator

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Table of contents

Content **Page**

List of Tables

Table **pages**

List of figures

List of abbreviations

CVDs	**Cardio Vascular Diseases**
BP	**Blood Pressure**
HTN	**Hypertension**
ACE	**Angiotesin Converting Enzyme**
DPPH	**1, 1-diphenyel-2-picrylhydrazyl**
EPHI	**Ethiopian Public Health Institute**
ROS	**Reactive Oxygen Species**
DBP	**Diastolic Blood Pressure**
SBP	**Systolic Blood Pressure**
MABP	**Mean arterial blood pressure**
PP	**Pulse Pressure**
W H O	**World Health Organization**
RAAS	**Rennin- Angiotensin- Aldosterone System**
SNS	**Sympathetic Nerves System**
ATR1	**Angiotensin Receptor1**
ATR2	**Angiotensin Receptor2**
SOD	**Superoxide Dismutase**
NO	**Nitrogen Monoxide**
NOS	**Nitric Oxide Synthase**
TNFα	**Tumor necrosis factor**

Abstract

Introduction:-Hypertension is a chronic elevation of systemic arterial pressure above a certain threshold value. It is a serious health problem worldwide that contributes to myocardial infarction, cerebrovascular accidents, and premature mortality. Medicinal plants are used to treat different diseases in different parts of the world. There are number of medicinal plants in the management of hypertension. One such plant is *Ruta chalpensis* which is used to treat different ailment in different parts of the world.

Objective: - The objective of this study was aimed to screen the secondary metabolites and to identify whether the aerial part of crude extract of *Ruta chalpensis* alters blood pressure and its effect on reactive oxygen species.

Materials and methods: -The study was conducted on twelve guinea pigs to measure the effect of *Ruta chalpensis* 70% ethanol extract by using invasive and organ bath methods. Qualitative chemical screening involving color change was applied to see the phytochemicals present in plant extract, and Spectrophotometric analysis by DPPH method has been used to see the antioxidant activity.

Result:- The areal part of 70% ethanol extract of *Ruta chalpensis* was found positive for, major secondary metabolites, The IC_{50}, value was computed to be 21.72mM of ascorbic acid and 58.20mM of 70% ethanol extract of areal part of *Ruta chalpensis* and an inhibition of 88.70% and 96.96% at 500ug/ml of the extract and the standard ascorbic acid respectively. After induction of aortic muscle contraction by high concentration of k^+ the extract showed a significant ($p<0.001$) ability to relax the isolated aorta of guinea pigs. The extract caused significant ($p<0.001$) fall in systolic, diastolic, and mean arterial blood pressure (MABP), There were no significant differences ($p=0.526$) in pulse pressure.

Conclusion: - This study justifies the traditional use of this plant for the treatment of hypertension and it can be additional source of natural antioxidant.

Key words: Hypertension; *in vivo*; *ex- vivo*; *Ruta chalpensis* Blood pressure; antioxidant; Phytochemical

1. INTRODUCTION

Hypertension is usually defined by the presence of a chronic elevation of systemic arterial pressure above a certain threshold value. However, increasing evidence indicates that the cardiovascular (CV) risk associated with elevation of blood pressure (BP) above approximately 115⁄75 mm Hg increases in a log-linear fashion(Giles *et al.,* 2009).

Hypertension, or high blood pressure, is a serious health problem worldwide(Benos, 2010).It is globally recognized as the most prevalent cardiovascular disease with potent complications such as coronary heart disease, stroke, sudden cardiac death, congestive cardiac disease, renal insufficiency and dissecting aortic aneurysm(Wichitsranoi *et al.,* 2011), and it also contributes to premature mortality and disability, and it highly affects populations in low and middle income countries where health systems are weak (WHO, 2013).

Hypertension can be classified into different categories on different basis; according to seventh report of joint national committee on prevention, detection, evaluation and treatment of high blood pressure (JNC7), Hypertension can be classified as; normal, pre-hypertension, hypertension $stage_1$, and hypertension $stage_2$. In this classification normal blood pressure(BP) is when systolic blood pressure(SBP) is <120mmHg and diastolic blood pressure (DBP) is < 80 mmHg, pre hypertension is when SBP is120-139mmHg or DBP is 80-89mmHg, $stage_1$ hypertension is when SBP is 140-149mmHg or DBP 90-99mmHg, $stage_2$ hypertension is when SBP is >160mmHg or DBP >100mmHg. According to British hypertension society (BHS), hypertension is classified as optimal, normal, high normal, $grade_1$ (mild), $grade_2$ (moderate), and $grade_3$ (severe). Optimal is when SBP is <120 and DBP is <80, normal is when SBP is <130 and DBP is <85, high normal is when SBP is 130-139 and DBP is 85-89, grade $_1$ or mild is 140-159SBP and 90-99 DBP, grade $_2$ or moderate type is when SBP is 160-179 and DBP is 100-109, and grade $_3$ or Sevier is >180 SBP and >110 DBP.

The other classification scheme for hypertension based on relative and absolute risk with implications for treatment and reimbursement uses, four basic ways to classify hypertensive individuals. The first uses only the level of BP (DBP, SBP) or both. Such a system is based on the increase in the relative risk (the proportional likelihood) of cardiovascular events occurring as BP rises. the second one is based on absolute risk, because, risks and ultimate outcomes in hypertensive individuals, are much more closely related to the presence of other cardiovascular risk factors and/or target organ damage than to BP level. Therefore, other systems based on absolute risk have emerged. A third type of system classifies hypertensive individuals on the basis of the expected benefit of treatment as demonstrated in clinical trials of antihypertensive therapy. The final way to classify hypertensive individuals would be to use some genetic, biochemical, or physiological parameter such as plasma rennin activity or the rennin-sodium index to denote those at high risk (Black, 1999). Therefore systems based on relative risk use of BP level alone, and systems based on absolute risk uses BP and/or co morbidity. Hypertension is already a highly prevalent risk factor for cardiovascular diseases (CVD) throughout the industrialized world. It is becoming an increasingly common health problem worldwide because of increasing longitivity and prevalence of contributing factors such as obesity, physical inactivity and unhealthy diet. The current prevalence in many developing countries, particularly in urban societies, is already as high as those seen in developed countries(WHO, 2003).

1.1 Types of hypertension

1.1.1Primary hypertension

This is also called essential hypertension is also known as primary or idiopathic hypertension, which comprises (95%) of high blood pressure causes. It has no clear underlying cause, but appears to be the result of interaction of complex genetic and environmental factors(Muhammad, 2009).

1.1.2 Secondary Hypertension.

Secondary hypertension comprises about (5%) of high blood pressure cases. It is caused by a specific underlying mechanism usually involving kidneys or endocrine system and also various conditions and medications can lead to secondary hypertension, including: kidney problems, adrenal gland tumors, thyroid problems, certain defects in blood vessels you are born with (congenital), certain medications, such as birth control pills, cold remedies, decongestants, over-the-counter pain relievers and some prescription drugs illegal drugs, such as cocaine and amphetamines, alcohol abuse or chronic alcohol use Obstructive sleep apnea, renal disease, genetic causes, renal vascular hypertension, primary hyperaldosteronism, Cushing's syndrome, pheochromocytoma, coarctation of the aorta (uncommon), hypertension associated with pregnancy, estrogen use, as well as other causes (e.g, hypocalcaemia and medications) (Karnath 2012; Muhammad Ilyas 2009).

1.2 Prevalence of hypertension

Nearly a quarter of the world's adult population is hypertensive. More than 75 million (one out of three) people in the United States alone are hypertensive, and an additional 50 million lie in the pre-hypertensive range(Wang *et al*., 2010). According to National Health and Nutrition Examination Survey, 2011–2012, the overall prevalence of hypertension among U.S. adults aged 18 and over was 29.1% and was similar among men (29.7%) and women (28.5%) (Nwankwo *et al.*, 2013) .

The prevalence of hypertension increased with age, from 7.3% among those aged 18–39, to 32.4% among those 40–59, to 65.0% among those 60 and over and the prevalence of hypertension was highest among non-Hispanic black adults (42.1%), compared with non-Hispanic white (28.0%), Hispanic (26.0%), and non-Hispanic Asian (24.7%) adults (Nwankwo *et al.*, 2013). According to American heart association statistical fact sheet 2013, a higher percentage of men than women have high blood pressure until age 45. From ages 45–54 and 55–64, the percentage of men and women is similar; after that a much higher percentage of women than men have high blood pressure. About 69% of people who have a first heart attack, 77% who have a first stroke, and 74% who have congestive heart failure have blood pressure higher than 140/90 mm Hg (Go AS *et al*., 2014).

High blood pressure was listed on death certificates as the primary cause of death of 61,762 Americans in 2009. In this year high blood pressure was listed as a primary or contributing cause of death in about 348,102 of the more than 2.4 million U.S. deaths (Wang and Scherer, 2008).

1.2.1 Prevalence of hypertension in Africa

The increasing prevalence of hypertension in developing countries is of great concern. According to a report from the World Health Organization (WHO, 2010), there was an estimated 972 million people with hypertension in the year 2000. 65% lived in developing world with the number predicted to grow to 1.5 billion by 2025(WHO, 2010).

Earlier reports also suggest that the prevalence of hypertension is rapidly increasing in developing countries and is one of the leading causes of death and disability. While mean blood pressure has decreased in nearly all high-income countries, it has been stable or increasing in most African countries. Estimates now show that in some settings in Africa more than 40 percent of adults have hypertension (epedemology of hypertension, 2013).

The prevalence of hypertension has increased significantly over the past two to three decades, there were approximately 80 million adults with hypertension in Africa South of the Sahara in 2000 and projections based on current epidemiological data suggest that this figure will rise to 150 million by 2025(Vijver *et al.,* 2013).

The WHO steps survey conducted between 2003 and 2009 in 20 African countries reported high rates of hypertension in most countries, particularly among men. The prevalence ranges from 19.3% in Eritrea to 39.6% in Seychelles. The prevalence is for the adult population aged 18 years and above. In Africa, hypertension is usually more pronounced in males than in females. However, in a few countries there were higher levels of prevalence in women than men such as in Algeria 31.6 % vs. 25.7 % in 2003, Botswana 37.0 % vs. 28.8 % in 2006 and Mali 25.8 % vs. 16.6 percent in 2007, for women and men, respectively (Vijver, 2013).

In South Africa and Democratic Republic Congo, the urban has almost 10 %point's higher prevalence than the rural population. This is in comparison to countries like Ethiopia and Tanzania where the prevalence is only a bit more than 5 percent higher. It is noteworthy that since countries are at different stages of the epidemiological transitions, there are some rural populations in some countries whose prevalence is higher than some urban populations in other countries (Vijver, 2013).

1.2.2 Prevalence of hypertension in Ethiopia

The epidemiology of hypertension in Ethiopia is not well studied. Some community-based surveys have shown that the prevalence of hypertension in the country varies from 1.8% in the rural community to 15 to 30% in urban areas of Addis Ababa and Gondar. Being obese or overweight, as well as physical inactivity, were strong predictors of hypertension in urban dwellers in Ethiopia (Gudina *et al*., 2013).

One community-based cross sectional study done in urban Addis Ababa showed that the age adjusted prevalence of high blood pressure was 31.5% among males and 28.9% among females. However, only 35.2% of the hypertensive subjects were aware of their high blood pressure and only 11% were on treatment with target blood pressure attained in 25.6%(Tesfaye *et al*., 2009).

A hospital-based cross-sectional survey conducted in southwest Ethiopia showed that the overall prevalence of hypertension was 13.2 % (Gudina *et al.,* 2013).Community based cross-sectional study among adults conducted in North West Ethiopia Gondar showed that the overall prevalence of hypertension was 28.3%, slightly lower in men (26.0%) than women(30.3%) though the difference was not statistically significant(Awoke *et al.,* 2012).

1.3 Pathogenesis of hypertension

Many biochemical processes are affected by hypertension and abnormalities or dysregulation of many biochemical pathways can contribute to the development of hypertension.Factors which influence blood pressure are, whole-body salt and volume load, autonomic nervous system output, lipid and cholesterol metabolism, kidney disease, medications, and lifestyle activities, such as smoking, drinking, and exercise (or lack thereof). There is a multitude of genetic influences as well (Benos, 2010).

Many causes have been implicated in the pathophysiology of hypertension such as; up regulation of the renin-angiotensin-aldosterone system, activation of the sympathetic nervous system, perturbed G protein-coupled receptor signaling, inflammation, and altered T-cell function. Common to these processes is increased bioavailability of reactive oxygen species (ROS) (termed oxidative stress) due to excess reactive oxygen species generation, decreased NO levels, and reduced antioxidant capacity in the cardiovascular, renal, and nervous systems (Vaziri, 2008).

1.3.1 Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone system (RAAS) is a signaling pathway responsible for regulating the body's blood pressure and fluid balance in the body. The interaction between the renin-angiotensin-aldosterone system and the sympathetic nerves system (SNS) is at least partially responsible for the development of hypertension(Manrique *et al.,*2010). RAAS regulates sodium balance, extracellular fluid volume, vascular resistance, and, ultimately, arterial blood pressure (Biala, 2011). It has been proposed that the RAAS has evolved in order to respond to changes in the level of blood volume and salt intake, which may occur under natural conditions(Karppanen and Mervaala, 2006).

The RAAS hormonal cascade begins with the biosynthesis of renin by the juxtaglomerular cells that line the afferent (and occasionally efferent) arteriole of the renal glomerulus. Renin is synthesized as a preprohormone, and mature (active) renin is formed by proteolytic removal of a 43-amino-acid prosegment peptide from the N-terminus of prorenin, the proenzyme or renin precursor. Mature renin is stored in granules of the JC cells and is released by an exocytic process involving stimulus-secretion coupling into the renal and then the systemic circulation.

Renin secretion is stimulated by a fall in perfusion pressure or in NaCl delivery, by an increase in sympathetic activity, and negative feedback by direct action of Angiotensin on the JC cells. It is also synthesized in other tissues, including brain, adrenal gland, ovary, and visceral adipose tissue, and perhaps heart and vascular tissue. Control of renin secretion is a key determinant of the activity of the RAAS (Atlas 2007).

Angiotensin has direct effects on renal vascular smooth muscle cells causing vasoconstriction of both afferent and efferent arterioles, resulting in the development of both glomerular capillary hypertension and reduced renal blood flow (Karppanen and Mervaala, 2006). Angiotensin II is a potent pro inflammatory agent that is able to modulate immune and inflammatory responses in endothelial, renal tubular and smooth muscle cells, such as chemotaxis, proliferation and differentiation of monocytes into macrophages (Pueyo *et al.*, 2000). Angiotensin II stimulates reactive oxygen species production by inducing vascular NADPH oxidase and ET-1 expression in the kidneys(Mennuni *et al.*, 2014a)

Angiotensin.II induces cardiac and vascular cell hypertrophy and hyperplasia directly by activating the Angiotensin II type 1 receptor and indirectly by stimulating release of several growth factors and cytokines. Activation of the Angiotensin receptor stimulates various tyrosine kinases, which in turn phosphorylate the tyrosine residues in several proteins, leading to vasoconstriction, cell growth, and cell proliferation, The activity of local RAAS and alternative pathways of Angiotensin II formation may make an important contribution to remodeling of resistance vessels and the development of target organ damage (including left ventricular hypertrophy, congestive heart failure, atherosclerosis, stroke, end-stage renal disease, myocardial infarction, and arterial aneurysm) in hypertensive persons(Mekoya, 2007).

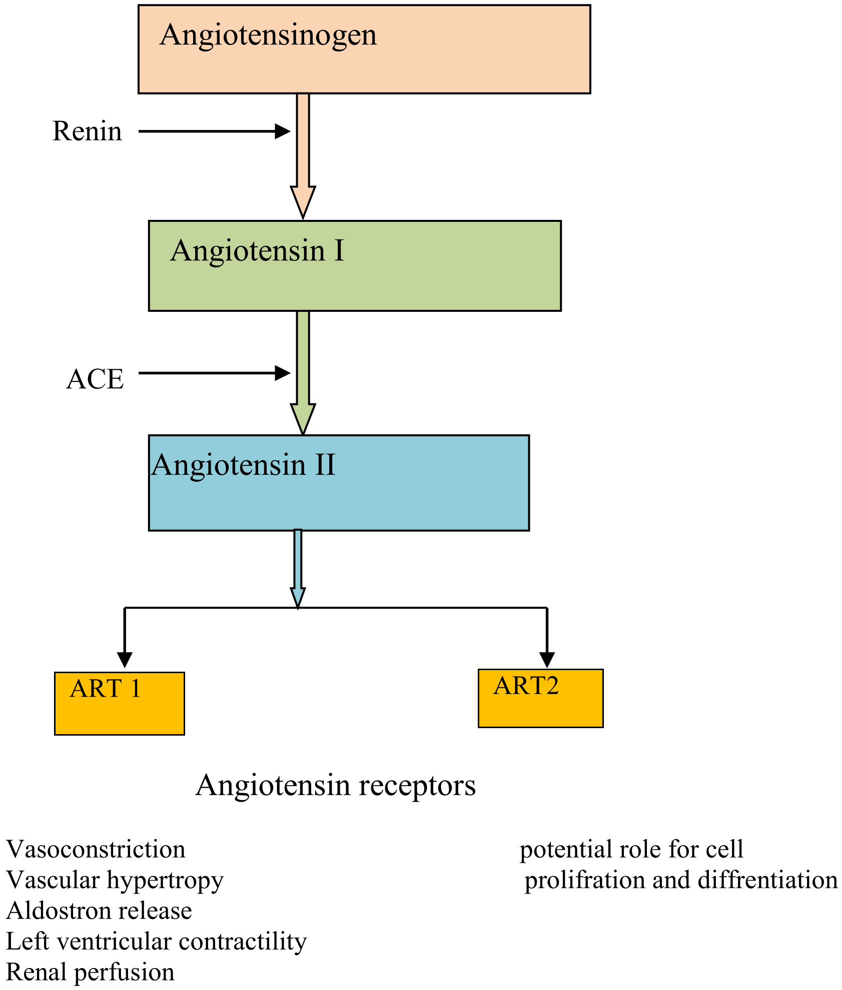


Figure1-1 Rennin angiotensin aldostron system (RAAS)(Naber *et al.*, 2004;Matsubara, 2000).

1.3. 2 Role of Oxidative Stress

Oxidative stress implies an increased production, or a decreased scavenging or metabolism of (ROS) (Wilcox, 2005; Mekoya, 2007).During normal metabolism in aerobic cells, molecular oxygen is reduced to water; yet the stepwise transfer of electrons generates free (ROS), including superoxide radicals ($O_2^{\cdot}$-), hydrogen peroxide(H_2O_2), and hydroxyl radicals($OH^{\cdot}$). Other radicals such as alkyl ($R^{\cdot}$), and peroxy ($ROO^{\cdot}$) radicals may also be produced endogenously, lipid peroxidationis also another significant source of reactive oxygen species (Brighente et al. 2007). Natural antioxidants such as vitamin E (α-tocopherol), vitamin c, and polyphenols or flavonoids are known to be used to treat and prevent oxidative stress (Nimmi, 2012; pal *et al.,* 2015).

Excess generation of reactive oxygen species and other radicals can damage proteins, carbohydrates, polyunsaturated fatty acids, and DNA, and may thus lead to oxidative stress and to a verity of degenerative processes and diseases such as aging, immunodeficiency, neurologic disorders, inflammation, arteriosclerosis, coronary heart disease, and certain cancers (Brighente *et al.,* 2007). Of the reactive oxygen species generated in cardiovascular cells, $O_2^-$and H_2O_2 appear to be particularly important (Paravicini and Touyz, 2008).

In biological systemsO_2^- is short-lived owing to its rapid reduction to H_2O_2 by superoxide dismutase (SOD) (Sharma *et al*., 2012)**.** The charge on the superoxide anion makes it unable to cross cellular membranes, except possibly through ion channels. In contrast, H_2O_2 has a longer biological lifespan than O_2^-, is relatively stable, and is easily diffusible within and between cells. The main source of H_2O_2 in vascular tissue is the dismutation of O_2:

$$2O_2 + 2\ H \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

This reaction can be spontaneous or it can be catalyzed by superoxide dismutase, (Sharma *et al.,* 2012)

The distinct properties between O_2^- and H_2O_2 and their different sites of distribution mean that different species of reactive oxygen species can activate different signaling pathways, which lead to divergent, and potentially opposing, functional responses. For example, increased O_2^- levels inactivate the vasodilator NO leading to endothelial dysfunction and vasoconstriction, characteristic of many vascular diseases, including hypertension on the other hand, H_2O_2 acts as a vasodilator in some vascular beds, including cerebral, coronary, and mesenteric arteries (Matoba *et al*., 2000).

Reactive oxygen species are produced by all vascular cell types, including endothelial, smooth muscle, and adventitial cells, and can be formed by numerous enzymes.

Enzymatic sources of reactive oxygen species that are important in vascular disease and hypertension are xanthine oxidase, uncoupled NOS, and NAD (P) H oxidase (Matough *et al.*, 2012).

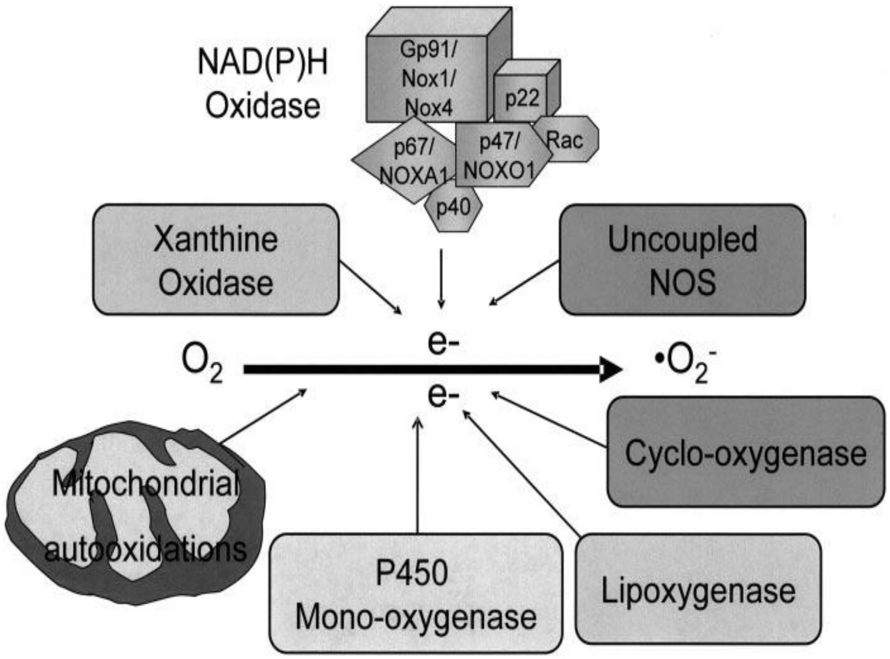


Figure 1-2: Enzymatic sources of superoxide anion (-O2 -).

The major enzymes responsible for reactive oxygen species generation in the vasculature include NAD (P) H oxidase, xanthine oxidase, and uncoupled NOS. NAD(P)H oxidase is a multisubunit enzyme, comprising gp91phox (or its homologs, Nox1 and Nox4), p22phox, p47phox (or NOXO1), p67phox (or NOXA1), and p40phox(Paravicini and Touyz, 2008).

Hypertension is associated with an elevation of reactive oxygen species and frequently also with an impairment of endogenous antioxidant mechanisms (Virdis *et al.*, 2011). Experimental manipulation of the oxidation reduction state *in vivo* shows that reactive oxygen species can be a cause of hypertension. The reactive oxygen species abundance in hypertension could result from increased production or impaired degradation of very active antioxidant enzymes, such as SOD and catalase, that convert superoxide to H_2O_2 and H_2O (Lassègue and Griendling, 2004).

In the cardiovascular system reactive oxygen species play a physiological role in controlling endothelial function, vascular tone, and cardiac function, and a pathophysiological role in inflammation, hypertrophy, proliferation, apoptosis, migration, fibrosis, angiogenesis, and rarefaction, all of which are important processes contributing to endothelial dysfunction and cardiovascular remodeling in hypertension (Verma *et al*., 2014; Raimondo *et al*., 2012)

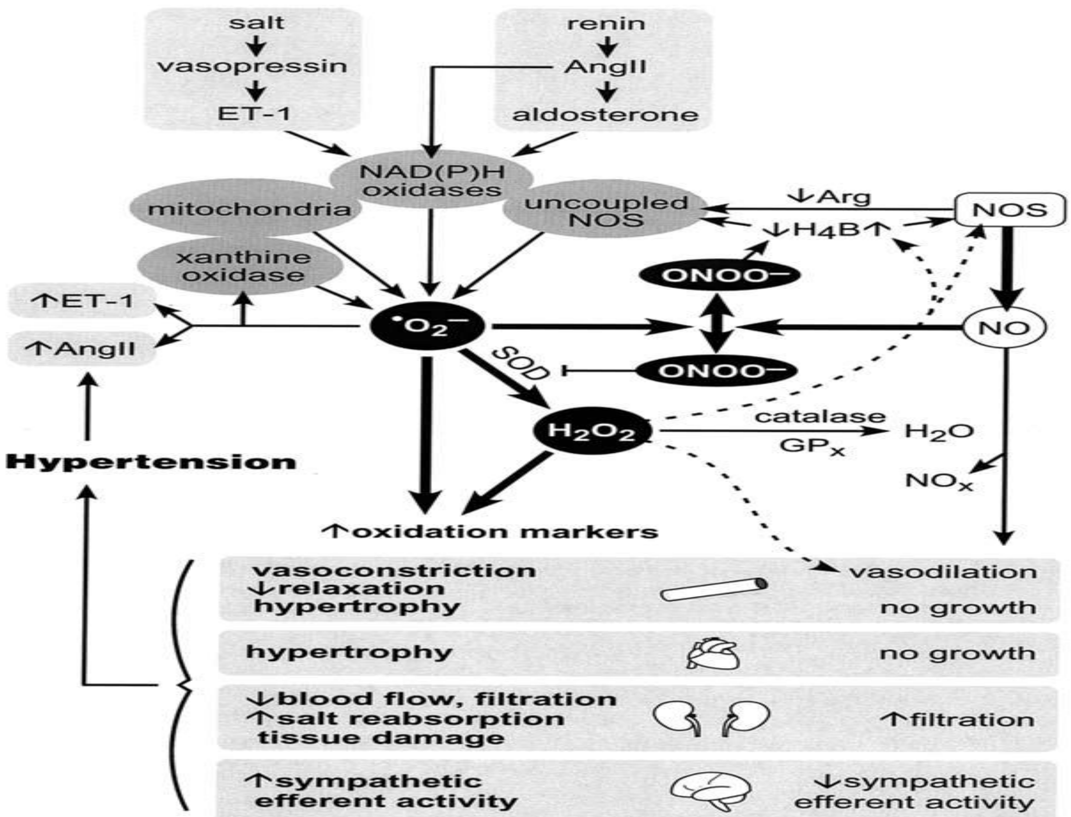


Figure 1-3: Major pathways leading to hypertension via reactive oxygen species formation(Mennuni *et al*., 2014b). Superoxide (O_2^-), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), endothelin-1 (ET-1), rennin, angiotensin II (AngII), angiotensin II (AngII).

Superoxide (O_2^-), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), endothelin-1 (ET-1), Reactive oxygen species, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$), are increased in target tissues, directly or indirectly, by hypertensive agents such as; salt, vasopressin, endothelin-1 (ET-1), rennin, angiotensin II (AngII), or aldosterone(Raimondo *et al.,* 2012)

Reactive oxygen species can diminish the efficiency with which the kidney uses O_2 for Na+ transport and thereby diminish the pO_2 within the kidney cortex. This may reduce reactive oxygen species generation, although it could further enhance vascular damage and hypertension. There is a tight relationship between renal oxidative stress and both development and maintenance of hypertension, many authors show that antioxidants can diminish not only the increase in blood pressure, but also the inflammation, fibrosis, sclerosis, and dysfunction of the heart, kidneys, and other organs of certain hypertensive models (Christopher, 2005).

Several enzymes as well as non enzymatic secondary metabolic compound of plant origin are able to scavenge reactive oxygen species and thus can protect the organism from oxidative damage (Alamgir *et al*., 2014).

1.3.3 Sympathetic Activation and Hypertension

Inappropriate and excessive activation of the sympathetic nervous system has been invoked as a cause of coronary heart disease (Esler, 2010). Increased sympathetic nervous system activity increase blood pressure and contributes to the development and maintenance of hypertension, through stimulation of the heart, peripheral vasculature, and kidneys, causing increased cardiac output, increased vascular resistance, and fluid retention (Oparil *et al*., 2003). Since most current evidence suggests that, in humans, sustained increases in heart rate are due mainly to decreased parasympathetic tone, these findings support the concept that autonomic imbalance contributes to the pathogenesis of hypertension (Oparil *et al*., 2003). In primary human hypertension, analysis of regional sympathetic nervous system function has demonstrated activation of the sympathetic nervous out flows to the heart, the kidneys, and skeletal muscle vasculature, particularly in younger patients, which contributes to blood pressure elevation (Esler, 2000).

1.3.4 Obesity and Hypertension

Obesity has rapidly become a major challenge to health care systems worldwide. According to the World Health Organization, more than 1.4 billion people worldwide were overweight and another 500 million were obese in 2008, and In the United States, approximately two-thirds of the adult population is overweight and nearly one-third is obese(Hall *et al.,* 2014).

Obesity is becoming recognized as one of the most important risk factors for the development of hypertension. Obesity may account for as much as 65% to 75% of human essential hypertension in most industrialized countries (da Silva *et al.,*2010).

Several epidemiological studies show that the age-adjusted prevalence of hypertension increases progressively with higher levels of body mass index (BMI) in men and women(Brown *et al*., 2000). Therefore, obese hypertensive women are at particular risk of developing left ventricular hypertrophy(Narkiewicz, 2006). Clinical studies showing that weight loss lowers blood pressure in normotensive or hypertensive obese subjects; and epidemiological studies showing that excess weight gain is a good predictor for development of hypertension (Jones *et al*., 2012).

Several mechanisms appear to be implicated in the development of hypertension associated with obesity, These include, alterations in the renin-angiotensin- aldosterone system, increased sympathetic nervous system activity, insulin resistance, leptin resistance, altered coagulation factors, inflammation and endothelial dysfunction are some of the mechanisms (Minor 2012; Hall 2014),obesity also causes excess renal sodium reabsorption, impaired renal-pressure natriuresis, and expansion of extracellular fluid volume, which lead to increased arterial blood pressure (Hall 2003; Hall *et al.,* 2010).

In general, there are three factors which are important in increasing renal sodium reabsorption, impairing pressure natriuresis, and causing the initial rise in blood pressure during rapid weight gain these are; increased sympathetic nerves system activity, activation of the renin-angiotensin- aldosterone system, and Physical compression of the kidneys by fat accumulation within and around the kidneys and by increased abdominal pressure due to accumulation of excess visceral fat, These mechanisms are closely interrelated and interact to raise blood pressure in obese subjects (Hall *et al.*, 2010)

1.3.5 Salt loading and Hypertension

There is evidence from observational epidemiological studies, small intervention studies and a controlled intervention trial as well as from studies in primates that dietary salt intake is one causal factor responsible for elevated BP in adults. This has also been shown in children (Kim *et al.,* 2014).

In humans, salt loading causes an acute increase in oxidative stress, particularly in individuals with salt-sensitive blood pressure. Salt loading not only increases blood pressure in salt sensitive individuals but in addition increases oxidative stress, and reduces nitric oxide availability by increasing the concentration of the eNOS inhibitor asymmetric dimethyl arginine (ADMA) (Piecha *et al.,* 2012).

Yet the mechanism by which dietary salt increases arterial pressure is not fully understood, but seems related to the inability of the kidneys to excrete large amounts of salt, studies also suggest chronic high salt intake increases cardiovascular morbidity and mortality both by its influences on blood pressure and by pressure independent effects on the blood vessels and heart(Meneton *et al.,* 2005).

Thus it seems beyond doubt that chronic high salt intake participates in the high prevalence of hypertension and cardiovascular disease in human population.

1.3.6 Primary Hyperaldosteronism (Conn's Syndrome)

Primary hyperaldosteronism is defined as overproduction of aldosterone independent of its usual regulator, the renin-angiotensin system (Angiotensin II) which is excess to the body’s requirement. This hormone is responsible for sodium and potassium balance, which then directly controls water balance to maintain appropriate blood pressure and blood volume(Stowasser et al. 2001). Recent studies have demonstrated that primary aldosteronism is the most common form of secondary hypertension when determinations of serum aldosterone, plasma rennin activity , and the serum aldosterone to plasma rennin activity ratio are used as screening tools (Mosso *et al*., 2003).

On the basis of the new data, which were obtained by determining the aldosterone/rennin ratio, the frequency of primary hyperaldosteronism in hypertensive(5-13%) is much higher than previously suspected (0.1-1%) (Beineke, 2010).New evidence indicates that 10% of patients with hypertension uncontrolled by more than one antihypertensive drug will have this condition and requires confirmation by measuring the blood ratio of aldosterone to renin(Beineke, 2010) .

There are two major types of primary hyperaldosteronism and three rare forms. The most common form is due to hyperplasia in both adrenal glands (about 60% of cases). A benign tumor of one of the adrenal glands is the cause in 35%. When the cause is a single adrenal tumor, it is labeled Conn's Syndrome. A malignant tumor is a very rare cause (Quinkler 2002; Pizzolo 2005). Primary hyperaldosteronism is a frequently neglected cause of residual hypertension despite technically successful endovascular treatment of renal artery disease (Pizzolo *et al*., 2005).

1.3.7 Coarctation of Aorta

Aortic coarctation is an obstruction of the aortic arch (Almeida & Pedersen 2012). Hypertension occurs with many patients with coarctation. The three theories which are responsible for the occurrence of hypertension and related with obstruction are mechanical (obstruction increasing arterial resistance), neural (obstruction resetting carotid baroreceptors), and renal (ischemia to the kidneys) (Prisant *et al*., 2004). Despite apparently successful correction of the obstruction, however individuals with a history of coarctation of aorta demonstrate excess morbidity and premature mortality associated with hypertension, cerebrovascular accident, coronary artery disease, aortic dissection/rupture, Stroke, heart failure, and sudden cardiac death (Hager *et al*., 2007) .

While blood pressure usually normalizes for a time after successful repair, one third of coarctation of aorta patients develop hypertension by adolescence and 90% by middle age(Hager *et al.,* 2007). Normotensive children and young adults who had undergone successful coarctation of aorta repair were found to have persistent endothelial dysfunction and impaired arterial reactivity, suggesting that intrinsic vascular abnormalities might contribute to the risk for premature Coronary artery disease (CAD) and hypertension (Bondy, 2012) .

1.3.8 Pheochromocytoma

Pheochromocytoma is a tumor of the chromaffin cells in the adrenal medulla and sympathetic paraganglia, which synthesizes and secretes catecholamines, Norepinephrine, epinephrine, and dopamine, they act on their target receptors, which causes a physiologic change in the body(Samuel, *et al*., 2012). Although the clinical presentation of pheochromocytoma is highly variable, approximately 95% of patients demonstrate hypertension and 50% have sustained hypertension (Zelinka, *et al*., 2005; Därr *et al*., 2012). Furthermore, pheochromocytoma is characterized by increased blood pressure variability, which constitutes an additional independent risk factor beyond increased blood pressure itself for cardiovascular morbidity and mortality (Calhoun *et al*., 2008) .

It is a life-threatening condition because catecholamine secretion is unpredictable, resulting in hypertension, arrhythmias, and/or hyperglycemia (Mekoya , 2007). The incidence of this disorder increases with advancing age, with the prevalence approaching 0.1% in elderly persons, and its prevalence is between 4% and 6.5% in patients who have an incidental adrenal tumor. There is also genetic predisposition to pheochromocytoma in between 10% and 20% of patients diagnosed with an apparent pheochromocytoma(Mekoya, 2007) .

1.3.9 Racial Differences and Hypertension

Etiology of hypertension is multi factorial and incidence, prevalence and mortality vary by race/ethnicity (Holmes *et al*., 2013). Prevalence of hypertension in African Americans is among the highest of any ethnic group in the world. Compared with whites, African Americans develop hypertension earlier in life and have higher average blood pressure. African Americans also have higher rates of more severe hypertension (180 or 110 mm Hg) than whites, causing a greater burden of hypertension complications (Bosworth *et al.*, 2006). A study has shown that the age-adjusted prevalence of hypertension by race, in the year 2003-2004, among the United States residents of age 20 years or older was 39.1% non-Hispanic Black, 28.5% non-Hispanic White, and 27.8% Hispanic, while age-unadjusted prevalence rate was 34.4%, 30.3%, and 16.9% for three racial groups, respectively(Holmes *et al.*, 2013).

1.4 Pharmacological agents available for treatment of hypertension

There are drugs which are used for the treatment of hypertension:
The first-line of treating hypertension is considered diuretics. Diuretics reduce blood pressure by inducing frequent urination. By urination, body is able to flush out the excess salt and water. And if this excess salt and water are not flushed out, these can cause an increase in blood pressure. When taking diuretics, there is the possibility that will experience some of its identified side effects like muscle cramps, dehydration, dizziness, extreme tiredness, skin rash, blurred vision, abnormal heart rate, and others(Reams & Bauer 2000).

Another type of anti-hypertensive medicine is angiotensin converting enzyme (ACE) inhibitors. Commonly known as angiotensin converting enzyme inhibitors, these medicines can cause dilation of the blood vessels which leads to an increase in the blood flow and a decrease in blood pressure. The following drugs are categorized as angiotensin converting enzyme inhibitors: fosinopril, benazepril, lisinopril, enalapril, captopril, and others. And like diuretics, angiotensin converting enzyme inhibitors also have certain side effects. Some of the side effects caused by angiotensin converting enzyme inhibitors are cough, kidney failure, skin rash, vomiting, diarrhea, fever, sore throat, and others(David, 2008).

Another anti-hypertensive drug is calcium channel blocker. Calcium channel blockers can cause widening of the blood vessels by blocking the entry of calcium into the cells of the heart and blood vessels. Just like with the other anti-hypertensive drugs, when blood vessels are dilated, there is increased blood flow and decreased blood pressure. The following medications are categorized as calcium channel blockers: verapamil, diltiazem, nifedipine, nicradipine, isradipine, felodipine, and amlodipine. Certain side effects come with the use of calcium channel blockers and examples of these are fatigue, headache, skin rash, diarrhea, constipation, edema, and others. The different side effects that come with the use of these medications are by far their biggest disadvantages. Scientific studies suggest different lifestyle changes and use of appropriate herbal medicine in the treatment of hypertension (Fraz, 2010).

1.5 Medical plants and their contribution to health

Plants have been an integral part of life in many indigenous communities, apart from providing building materials, fodder, weapons and other commodities, plants are especially important as traditional medicines (Bussmann *et al.,* 2011). A large number of people in both developing and developed countries rely on medicinal plant products to maintain their health or treat illnesses (Street and Prinsloo, 2013). Medicinal plants, defined as plants used for maintaining health and/or treating specific ailments, are used in a plethora of ways in both allopathic and traditional systems of medicine in countries across the world. Even people using only allopathic medicine throughout their lives are likely to be somewhat medicinal plant reliant as 20-25% of drugs prescribed are plant derived (Street and Prinsloo, 2013).

About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organization (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors (Rates, 2001). Examples of important drugs obtained from plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthusroseus*, atropine from *Atropabelladona* and morphine and codeine from *Papaversomniferum*. It is estimated that 60% of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin (Rates, 2001).

Before the advent of western medicine, indigenous people worldwide have been taking care of their health needs using plants. The African continent has a long history of the use of plants and in some African countries up to 80% of the rural population relies on medicinal plants as a source of remedies(Henry *et al.,* 2013). Medicinal plants have always had great significance in culture; medicine and nutrition of societies in the world (Street and Prinsloo 2013; Garbers and Dubois, 1999). Surveys of plant medicinal usage by the American public have shown an increase from just about 3% of the population in 1991 to over 37% in 1998, the north American market for sales of plant medicinal has climbed to about $3 billion/year (Briskin, 2014).

It is believed that about half of the top 25 best selling medicines in the world originate from natural materials, including plant substances(Masayoshi Shigeta, 2008). On top of their use in fighting various ailments at local level, different medicinal plants are used as export commodities, which generate considerable income. China takes the lead (45%) by importing the highest number of herbal medicines for preparation of drugs and this is followed by the United States of America (15.6%) and Australia (10.5%) (Megersa *et al*., 2013).

1.5.1 Medicinal plants in Ethiopia

The medicinal plant of Ethiopia and the developing countries play major supplementary roles to the limited modern health care available (Lulekal *et al.,* 2013), and majority of Ethiopians rely on traditional medicine as their primary form of health care, yet they are in danger of losing both their knowledge and the plants they have used as medicines for millennia (Avigdor *et al*., 2014).

Ethiopia has been described as one of the most unusual and important sources of biodiversity in the world (Avigdor *et al*., 2014), and traditional medicine still remains the main resource for a large majority of the people in Ethiopia for treating health problems (Teklehaymanot and Giday, 2007).

The various literature available show the significant role of medicinal plant in primary health care delivery in Ethiopia where 70% of human and 90% of livestock population depend on traditional medicine again similar to many developing countries particularly that of Sub-Saharan African countries (Bekele, 2007). The wide spread use of traditional medicine in Ethiopia could be attributed to cultural acceptability, efficacy against certain type of diseases, physical accessibility and economic affordability as compared to modern medicine (Megersa*et al.,* 2013). Medicinal plants found in the Ethiopian flora were at one time estimated to be over 700 species while in a later communication about 1000 identified species were included (Mesfin *et al.,* 2013; Belayneh *et al.*, 2012).

The flora of Ethiopia is diverse with about 6,500-7000 species of higher plants with about 12% endemic elements. Among African countries, Ethiopia is often quoted as one of the six countries of the world where about 60% of the plants are said to be indigenous with their healing potential (Regassa, 2013).

1.5.2 Role of medicinal plant in the treatment of hypertension

Use of alternative medicine is common in the management of hypertension which is one of the most common non-communicable diseases worldwide affecting up to 800 million(20%) of the world's adult population (Osamor and Owumi, 2010).

A study conducted on medicinal plant which is used to treat diabetes and hypertension in Cameron a total of 182 patients who have been diagnosed at least once by a physician with diabetes and/or hypertension were selected and the selected 182 patients took different varieties of medical plants for a period of 10 days. These medicinal plants were prepared by boiling and administered orally twice a day. As a result, 70% of patients were diabetes and/or hypertensive free at the end of 10 days treatment (Tsabang *et al*., 2014).

More than 100 Medicinal herbs from different families are used for treating hypertension these include; A. *cepa*, *A. sativum*, *O. europea*, and *P. verticillata*. *Olive europaea, Hibiscus species, Citrus limonum, Urticadioica, Carica papaya, Cymbopogon Citrates, Aloe vera, Nigella sativa* (Vejendla *et al.,* 2011), Conventional medicines usually treat the symptoms of high blood pressure but seldom address the underlying causes. In modern medical society, several drugs and treatment regimen have developed to treat diseases. Although they obtain positive impacts, yet there are certain limitations encountered in the management of the disease due to their adverse effects and non-compliance by the patients (Zar and Das, 2013).

1.6 *Ruta chalepensis* L. (Family-Rutaceae) Its medicinal property and uses

Rue (*Ruta chalpensis*) is a genus of strongly scented evergreen sub shrubs 20–60 cm tall, in the family Rutaceae, distributed in temperate and tropical countries. The genus name "Ruta" comes from the Greek word "reuo ", to set free, showing its reputation as a free from disease. There are perhaps 8 to 40 species in the genus. A well-known species is the *Rue*. The leaves are bipinnate or tripinnate, with a feathery appearance, and green to strongly glaucous blue-green in colour. The flowers are yellow, with 4–5 petals, about 1 cm diameter, and borne in cymes. The fruit is a 4-5 lobed capsule, containing numerous seeds (kumar, 2011).

Figure 1-4: Aerial part of *Ruta chalepensis* (a photograph taken from botanical garden of EPHI)

Ruta chalepensis is known to be used as laxative, anti-inflammatory, analgesic, antipyretic, abortifacient, anti-epileptic, anti-helmintic, and for dermatopathy treatmen(Beatriz *et al*.,2010).

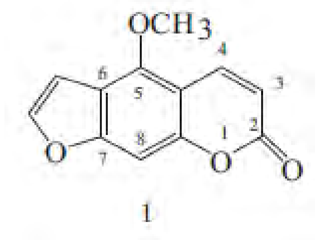
It has also many biological activities such as molluscicidal activity, larvicidal activity, and repellent activity, previous phytochemical research on this plant has resulted in the isolation of alkaloids, coumarins and flavonoids and it is a rich source of important secondary metabolites such as furanocoumarins and alkaloids. Besides, it is a medicinal plant and still used in traditional medicine (Günaydin a and Savci b † 2005; Tsabang *et al.,* 2014).

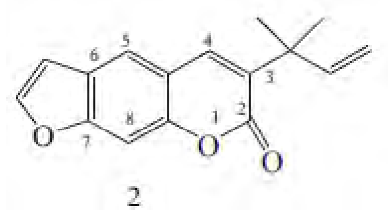
Ruta chalepensis is claimed in folk medicine and used in Mexico for treatment of hypertension and other cardiovascular disorders. The data obtained from this study suggest that the aqueous extract of *Ruta chalepensis* leaves has antihypertensive effect, and support its use as an antihypertensive agent in traditional medicine (Beatriz *et al*., 2010).

Crude extracts of *Ruta chalepensis* showed antfertility activity in female rats, an ethanolic extract of the aerial parts of *Ruta chalepensis* was studied for its anti-inflammatory, antipyretic, analgesic and CNS depressant activities, in Saudi Arabia, decoction of the aerial parts of the plant is used as an analgesic and antipyretic and for the treatment of rheumatism and mental disorders. The plant is prescribed in the Indian system of medicine for the treatment of dropsy, neuralgia, rheumatism and menstrual and other bleeding disorders. In China, a decoction of the roots of the plant is used as ant venom.

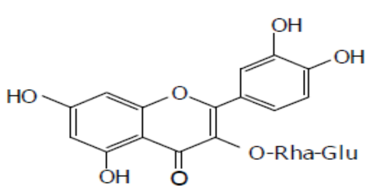
The leaves of this plant infused with vinegar are given to children for the treatment of convulsions and other nervous disorders. An aqueous decoction of the leaves is used for the treatment of fevers in Africa (Günaydin a and Savci b † 2005), in Ethiopia it is used as a spice, as a flavoring in tea, coffee and milk, and medicinally it is used to treat common cold, stomachache, diarrhea, influenza (Gall *et al*., 2009).

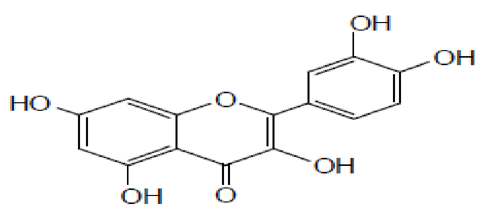
The following are chemical structures of the isolated compounds from the aerial parts of *Ruta chalepensis*





Bergabten   Chalpensin





Rutin   Quercitn

Figure 1-5: Chemical structures of the isolated compounds from the aerial parts of *Ruta chalepensis*(Shehadeh *et al.,* 2007; Nuengchamnong *et al.,* 2004)

1.7 Statement of the problem

The economic burden of cardiovascular diseases in Africa is significant and the diseases will cost the continent billions of dollars in the next decade. Hypertension remains the number one cause of significant financial burden, including the cost of caring for all the complications arising from it like stroke, ischemic heart disease and congestive heart failure. The financial burden comes in the form of direct healthcare costs related to treatment of cardiovascular diseases and its risk factors.

Although there is availability of low-cost therapy for hypertension, more effective and better tolerated antihypertensive therapies will be needed, of which natural products can be considered as one of the potential sources. It is well known that the study of medicinal plant species had allowed the isolation of several agents, used as leads for the development of new therapeutic drugs. Scientific evidence from tests done to evaluate the safety and effectiveness of traditional medicine products and practices is limited. While evidence shows that some herbal medicines and some manual therapies (e.g. massage) are effective for specific conditions, Therefore the aim of the present study is to investigate the antihypertensive effect of traditionally used herbal remedy(*Ruta chalepensis)*,the secondary metabolites present in it and its effect on the reactive oxygen species (ROS).

1.8 Significance of the Study

Hypertension is a major risk factor for cardiovascular disease, and the latter is the leading cause of morbidity and mortality worldwide. In developed countries, hypertension ranks as the top contributing factor for mortality and third in causing disability(WHO, 2009). Hypertension is estimated to cause 4.5% of the global disease burden and is as prevalent in many developing countries as in developed countries. Worldwide; seven million premature deaths have been attributed to hypertension. In recent decades, it has become increasingly clear that the development of stroke, ischemic heart disease, and renal failure have been attributed by hypertension(Penang *et al.,* 2010).

In the context of the epidemiological transition in Ethiopia, a double burden of disease is already emerging with the mix of persistent infectious diseases and increasing non communicable diseases and injuries. The prevalence of non communicable disease is increasing, owing to lifestyle changes. Among these diseases, hypertension is the seventh leading cause of mortality(WHO, 2010).

Therefore it is very important to study the antihypertensive or hypotensive activity of medicinal plants, since many developing countries like Ethiopia in which the majority (70-80%) of population depend on medicinal plants or traditional medicine.

1.9 Hypothesis of the study

This study hypothesizes that, the crude extract of areal part of *Ruta chalpensis* has pressure lowering effect, when the extract is administered intra venous and it has also antioxidant activity because of the secondary metabolite present in it.

2. Objectives of the Study

This study was aimed to attain the following general and specific objectives.

2.1 General Objectives

- To identify whether the areal part of 70% ethanol extract of *Ruta chalpensis* alters blood pressure, its effect on isolated aortas of guinea pigs and on reactive oxygen species.

2.2 Specific Objectives

- To perform phytochemical screening of areal part 70%ethanol extract of *Ruta chalpensis* to identify the major secondary metabolites.
- To examine the *in vivo* hypotensive effect of crude extract of areal part of 70%ethanol extract *of Ruta chalpensis* in guinea pigs.
- To study the relaxation effect of aerial part of 70% ethanol extract of *Ruta chalpensis* crude extract in isolated aorta of guinea pigs.
- To determine dose dependent effect and efficacy of the extract in guinea pigs.
- To study the effect of free radical inhibitory activity of aerial part of 70%ethanol extract of *Ruta chalpensis.*

3. Materials and Methods

3.1 Study setting

The Study was conducted at Addis Ababa University, school of medicine, laboratories of biochemistry (dose preparation and measurement), Physiology (experiment on guinea pigs), pharmacology, and Ethiopian public health Institute (EPHI) (for antioxidant activity and phytochemical screening) on .

3.2 Study design

The study design was randomized experimental study.

3.3 Plant collection and authentication

Sufficient amount of areal part of *Ruta chalepensis* was collected from different households around gulelle sub city Addis Ababa, which is located in northern suburb of the city, it borders with the districts of kolfe keranio, Addis ketema, Arada and Yeka, and the plant was collected at flowering stage and the plant was identified by botanist in the department of Traditional and Modern Medicine Research Directorate of Ethiopian public Health Institute (EPHI) with the voucher number 20/1985.

3.4 Extraction methods

350 gm of fresh plant material were dried under shade. Once thoroughly dried, the plant materials were grinded using mortar and cement, and the powdered plant weighing 23.53gm were macerated by 70% Ethanol for 72 hour. The extract were filtered by using whatman No 1 filter paper, and the filtrate was evaporated under reduced pressure at 37^0C water bath by using rotary evaporator and finally the crude extract were obtained.

3.5 Phytochemical screening

The plant extract were subjected to phytochemical screening technique to identify the major secondary metabolites using qualitative chemical test involving color changes through reaction with different standard reagents (Prashant, 2011).

3.5.1 Antioxidant activity

The antioxidant activity of the 70 % ethanolic extract was examined by comparing it with that of the known antioxidant compound, ascorbic acid, by the assay of scavenging stable free radical, 1,1-diphenyl-2-picrylhydrazyl(DPPH) according to Brighente and his co workers, 2007.

3.5.2 DPPH assay

The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Salvekar, 2011), DPPH is converted to 1, 1-diphenyl -2-picryl hydrazine when it reacts with antioxidants and a change in colour from purple to yellow is observed. The diluted working solutions of the test extracts and the standard were prepared in methanol. Ascorbic acid was used as standard in 7.88-500 μg/ml concentration range. 0.004% of DPPH was prepared in methanol and 2 ml of this solution was mixed with 2 ml of sample solution and standard solution separately. These solution mixtures were kept in dark place for 30 min. the control contains only DPPH solution in methanol while methanol served as a blank (negative control). The reduction capability of DPPH radical was determined by the decrease in its absorbance. Absorbance was noted at 517nm by using UV-VIS spectrophotometer (Brighente *et al*., 2007). The percentage of DPPH scavenging by the extract and standard compound were calculated as follows;

$$\% \text{ Inhibition} = [(A_0 - A1)/A_0] \times 100$$

A_0: absorbance of the control and A_1: absorbance in the presence of the sample of extract and Standard.

Laboratory Animal Preparation and Experimentation

Twelve male guinea pigs (300 to 600g) were obtained from Ethiopian public Health Institute, (EPHI) Addis Ababa, Ethiopia, and placed in the animal house of the Department of Pharmacology, Collage of Health Science, Addis Ababa University. The animals were housed with water and food *ad libitum*, ambient temperature of 22 ± 2°C and 50 ± 10% relative humidity with 12hrs light and 12hrs dark cycles and they were given one week acclimatization period.

3.7 Procedure for measuring guinea pig blood pressure:

3.7.1 *ex- vivo* experiment on guinea-pig aorta using organ bath

The *ex-vivo* experiment was conducted according to the method described by (Ghayur & Gilani 2005; Gilani *et al.*, 1999; Gilani *et al.* 2010). Six guinea-pigs of either sex (400- 600g) were sacrified and the descending thoracic aorta was quickly removed and placed in Krebs-Henseleit solution. Excess fat and connective tissues were trimmed off and the whole length of aorta was then cut spirally resulting in long strip, from this strip a short strip (2 to 4 cm) was prepared to be used for the experiment. The tissue was kept moistened with Krebs-Henseleit solution during the whole procedure and the strip preparation was mounted in a 2.5ml tissue bath containing Krebs–Henseleit solution, maintained at 37 ^{0}C and continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide.

The composition of the physiological salt solution was (g/L): (NaCl= 6.9, $NaHCO_3$= 2.1, $CaCl_2$ =0.36, KCl =0.373, KH_2PO_4= 0.16, $MgSO_4$ =0.141 and glucose 2, pH 7.4. A resting tension of 1g was applied to the tissue and an equilibrium period of 1hr and 10 minutes was allowed to equilibrate before addition of any drug or the test extract. During this period the bath fluid was changed every15minutes. Effect of extract was first determined on the resting baseline of the tissue to see if it had any vasoconstrictor effect. High K^+ (80 mM) was added in the bath to induce sustained contraction of the tissue. The extract was later tested for its ability to inhibit (relax) the contraction induced with high K^+ (80 mM). Changes in isometric tension of the strip was measured via a force displacement transducer (FT- 03) using a grassmodel 7E polygraph (Grass Instrument Co., Quincy, Mass., USA)

3.7.2 *In vivo* experiment using invasive method

The *in vivo* experiment was carried out according to the method described by previous researchers (Ghayur & Gilani 2005; Gilani *et al.*, 1999; Gilani *et al.* 2010) on six male guinea pigs (300 - 600g) anaesthetized with pentobarbital (60 mg/kg, i.p.).The reflex of the animal are checked, the skin on the ventral side of the neck and chest is carefully shaved and disinfected, and is placed on a rodent surgical table. The trachea was exposed using a scalpel blade and cannulated and the animals were artificially ventilated to facilitate spontaneous respiration (Bioscience, 815-51190-1, Sheemess, Kent, UK).

The arterial blood pressure was recorded from the carotid artery filled with heparinized saline via an arterial cannula (Portexcannulae, external diameter 1.02 mm, internal diameter 0.75 mm) connected to a pressure transducer. The extract of *Ruta chalpensis* and the drugs were injected in the form of bolus injection via a cannula (Portexcannulae, external diameter 1.02 mm internal diameter 0.75 mm) inserted into the external jugular vein followed by saline flush (0.2 ml). The exposed surface for cannulation was covered with cotton wool moistened in warm saline. The temperature of the animal was maintained at 37^0C by the use of a heated table and overhead lamp. The animal was then allowed to equilibrate for at least 30 min before administration of any extract.

The pressure transducer was connected to a polygraph or BBC recorder to measure systolic and diastolic blood pressure. Pulse pressure was obtained by subtracting diastolic pressure from systolic pressure and mean arterial blood pressure (MABP) was also determined from the sum of DBP plus one-third of pulse width. Changes in blood pressure were expressed as the Mean ± Standard error of the means of control values, obtained before administration of test substances.

3.7 statistical tests

The data for the systolic, diastolic pressure and duration of action for the extract was calculated form calibration scale that was made at the beginning of each experiment. Pulse measure was taken as the difference between systolic and diastolic pressure. Mean blood pressure was calculated as diastolic pressure plus one-third of the pulse pressure.

The data statically were compared by repeated measures using ANOVAs, two tailed student's t-test and independent t-test. The data were expressed as mean and means plus or minus standard deviations of the numbers of experiments.

4. RESULTS

4.1 Phytochemical Screening

The extracts of the aerial part of *Ruta chalpensis* was analyzed for the presence of various phytochemical constituents like chromophers, Tannins, phenols(Ferric chloride test) Saponnins(Foam test), Flavonoids(Lead acetate test), Terpenes/steroids (Liebermann - Burchard's Test), Alkaloids(Mayer's Test, dragendroff's test), Anthraquinones (Borntrager'stest), and cardiac glycoside(Keller reaction), using standard phytochemical procedures, and it is found strongly positive for, chromophors, polyphenols, flavnoids, and alkaloids and it is positive for saponins, phytosteroids and withanoids,and tannins and it is slightly positive for antraqunon glycosides and cardiac glycosides.

Table 1: Results of preliminary phytochemical analysis of crude extract of *Ruta chalpensis with* the characteristic color indicators.

No	Secondary metabolite	Indicators	Result
1	Chromophores	Yellow to red color	++
2	Polyphenols	Green blue color	++
3	Saponins	Formation of persistent honey comb forth	+
4	Phytosteroides andWithanoids	Formation of red, reddish brown, or violate color	+
5	Flavonoids	Development of yellow or orange color	++
6	Tannins	Formation of color	+
7	Alkaloids	Yellow orange ppt	++
		Whitish ppt	+
8	Antraquinone Glycosides	Pink, red, or violet color in ammonium phase	+/-
9	Cardiac glycoside	Thick blue color	+/-

Concentrations: ++high, +medium, +/- slight

As it was observed in Table 4.1 there were alkaloids, chromophores, polyphenols, saponins, phytosteroides, withanoids, flavonoids and tannins, in qualitative, chemical screening of *Ruta chalpensis* 70% ethanol extract.

4.2 Result of antioxidant activity

The most widely used assays for screening antioxidant activity of plant extract is DPPH radical scavenging activity. Table 4.2 shows the antioxidant activity of the 70% ethanol extract of *Ruta chalepensis* aerial part assessed using the DPPH radical scavenging activity. Moderate to high DPPH scavenging activity were recorded at a concentration between 7.88 - 500 µg/ml of 70% ethanol extract of *Ruta chalepensis*

Table 2: measurment of absorbance at 517nm and Percent Inhibition effect of the standard Ascorbic Acid and the extract from concentration range of 500-7.88ug/ml,

concentration(ug/mL)	Absorbance of (Vit C)	Absorbance (A) of extract	% inhibition extract	% inhibition Standard(Vit C)
7.88	3.390	2.723	22.70792	3.775192
15.625	2.280	2.025	42.52058	35.28243
31.15	0.322	1.235	64.94465	90.86006
62.5	0.120	0.842	76.09991	96.59381
125	0.110	0.689	80.4428	96.87766
250	0.109	0.421	88.04996	96.90605
500	0.107	0.398	88.70281	96.96282

The IC_{50} which is concentration required to quench 50% of the DPPH radical, was computed to be 21.72mM of ascorbic acid and 58.20mM of 70% ethanol extract of *Ruta chalpensis* and an inhibition of 88.70% and 96.96% at 500ug/ml of the extract and the standard ascorbic acid respectively.

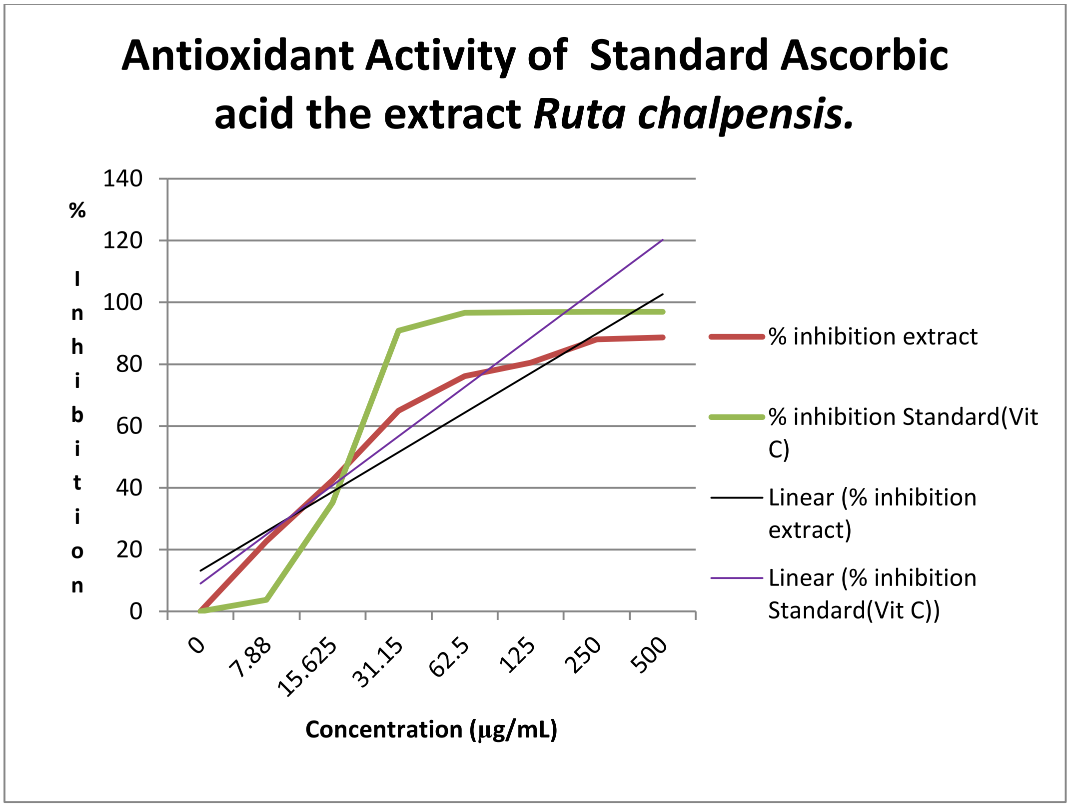


Figure 4-1: Free radical scavenging activity of 70%Ethanol extract of *Ruta chalpensis* using DPPH Assay.

4.3 Result of blood pressure studies in guinea pigs

The effect of 70% ethanol extract of aerial part of *Ruta chalpensis* on systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure, and mean arterial blood pressure (MABP) in anesthetized guinea pig (n = 6)

4.3.1 Result of *ex vivo* experiments on isolated guinea pig aortas

For *ex vivo* experiments, to achieve a baseline contraction (100%) of guinea pig aortas, the tissues were treated with high potassium chloride concentrations (K^+=80mM). Mean percentage relaxation of guinea pigs aortas treated with various doses of *Ruta chalpensis* extracts (2, 4, 6, 8 and 10 mg/mL) is shown in (Figure 4.2). The mean percentage relaxation of guinea pig aorta treated with 2 and 4 mg/mL of extract was found to be 10.88 ± 2.87% and 27. 15 ±4.20 %, it was not significantly relaxed ($p=0.807$), for treated with 2 mg/mL and ($p<0.94$) for treated with 4 mg/mL as compared with the baseline contraction.

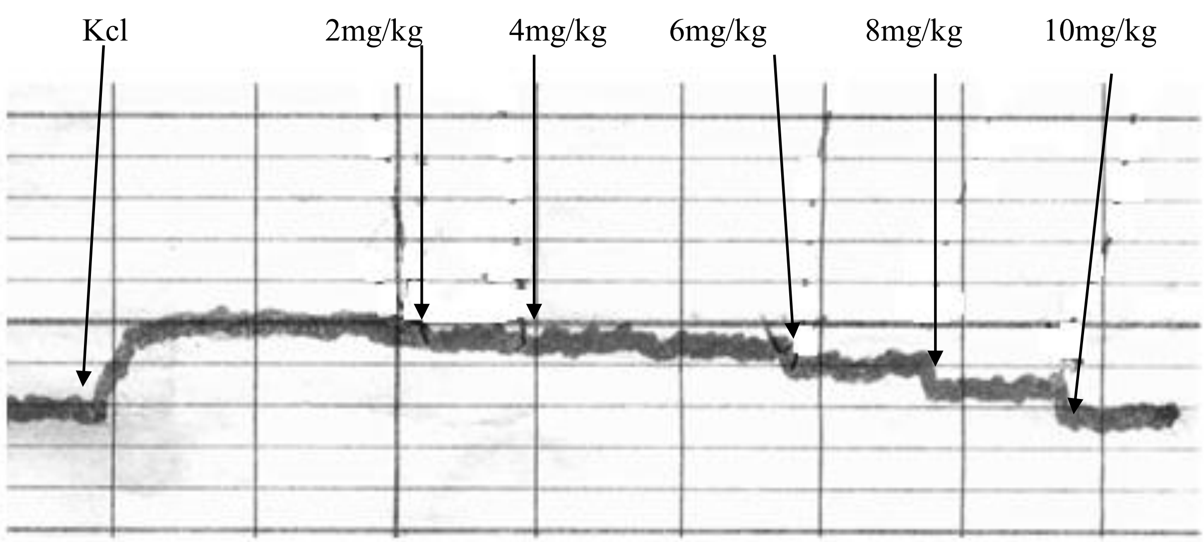


Figure 4-2: Typical tracing of relaxation of guinea pig aorta by *Ruta chalpensis*

However, treatment of isolated aortas with increasing doses of extract caused significant relaxation of aortas. Percentage relaxation of aortas at higher doses of extract was; 47.96 ± 4.97 % for 6 mg/kg extract; 66.12 ± 9.92 % for 8 mg/kg extract; and 83.47 ± 15. 57 % for 10 mg /kg extract), ($p<0.001$ for extract doses 6 mg/kg, 8mg/kg and 10 mg/kg extract). This was dose-dependent, with higher doses of extract causing progressively greater relaxation of aortic muscle than lower doses (Figure 4.2).

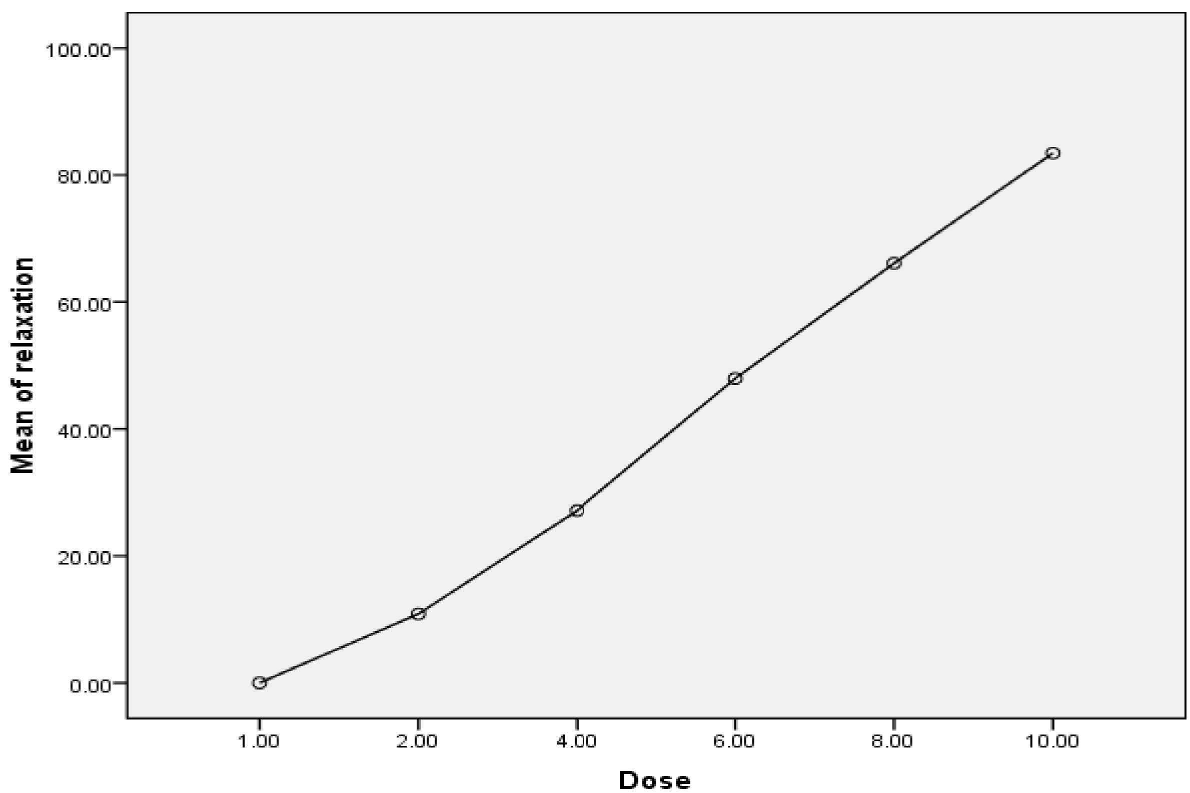


Figure 4-3: The effect of *Ruta chalpensis* in guinea pigs aorta

4.3.2 Effect of extracts of *Ruta chalpensis* aerial part *in vivo* on guinea pig blood pressure

The effect of 70% ethanol extracts of aerial part of *Ruta chalpensis* on the systolic blood pressure in guinea pigs before and after treatment with various doses (12, 26, 42, 60 and 80 mg/kg) of extract injected into the jugular vein was examined. The mean systolic blood pressure of guinea pigs treated with each dose of extract tested was significantly ($p<0.001$) lower than guinea pigs before treatment (Figure 4.5). At extract dose of 12 mg/kg, systolic blood pressure was reduced from 84.54167 ± 0.71 mm Hg to 65.33± 1.92 mmHg and, higher doses of *Ruta chalpensis* extract cause increasingly higher reductions in systolic blood pressure (Figure 4.5). Thus, 80 mg/kg extract reduced systolic blood pressure from 84.54 ± 0.71 mmHg to 58.83 ± 3.87 mmHg.

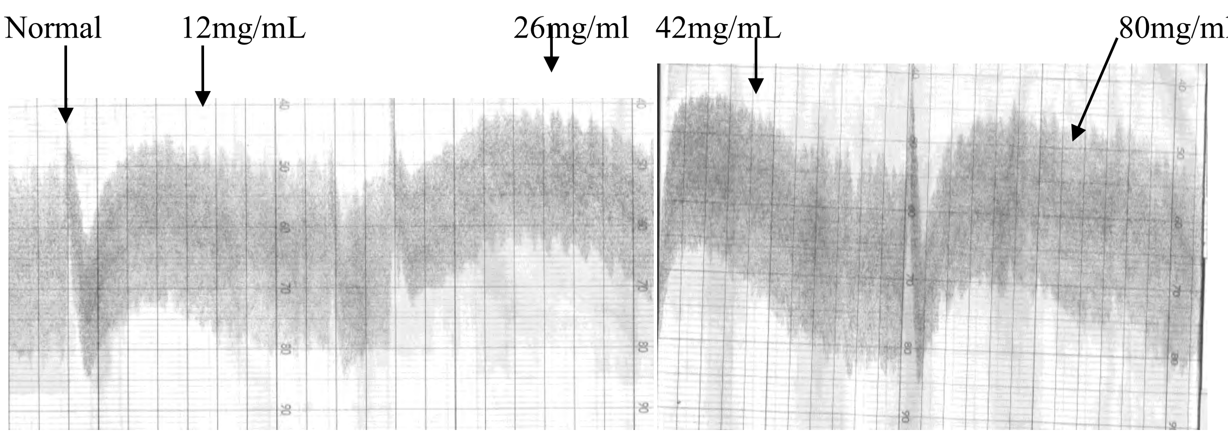


Figure 4-4: Typical tracing of blood pleasure measurement of guinea pig by *Ruta chalpens*

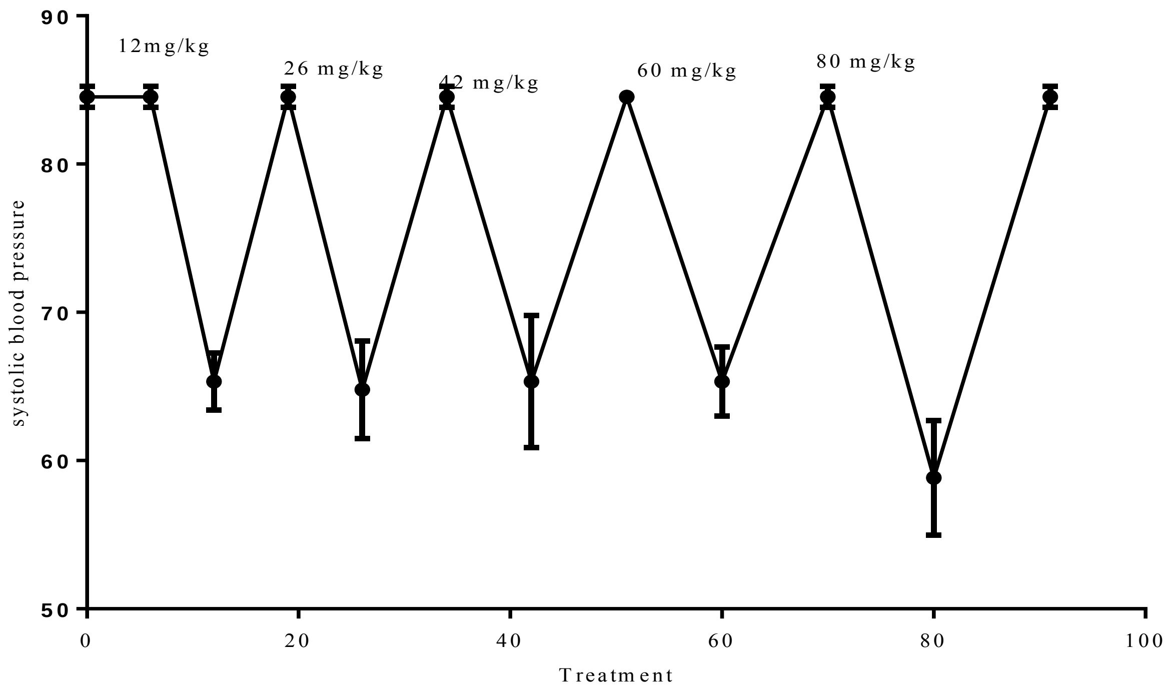


Figure 4-5: Effect of *Ruta chalpensis*on systolic blood pressure in guinea pigs

A group of six guinea pigs were treated intravenously (via external jugular vein) with increasing doses (12, 26, 42, 60, 80 mg/kg) of extract of *Ruta chalpensis* aerial part. Blood pressure was continuously monitored via a transducer connected to the carotid artery. After each treatment with extract, blood pressure was allowed to return to baseline prior to treatment with the next dose of extract. Comparison was done with paired t-test and $p<0.05$ was considered significant.

The effect of extract on the diastolic blood pressure (DBP) of guinea pigs before and after treatment with various doses (12, 26, 42, 60 and 80) mg/kg of extract given through jugular vein was examined. The mean diastolic blood pressure of guinea pigs treated with each dose of extract was significantly ($P<0.001$) lower than the diastolic blood pressure of the guinea pigs before treatment with extract (Figure 4.6). Mean DBP of guinea pigs before treatment was 60.56 ± 1.68 mm Hg, whereas treatment with 12 mg/kg extract lowered DBP to a mean of 54.83 ± 4.17 mmHg, and treatment with increasing doses of extract caused progressively higher drops in DBP, showing a dose-dependent effect (Figure 4.6). Thus, 80 mg/kg extract caused a drop in DBP from 60.56 ± 1.68 mm Hg to 39.50± 2.70 mmHg.

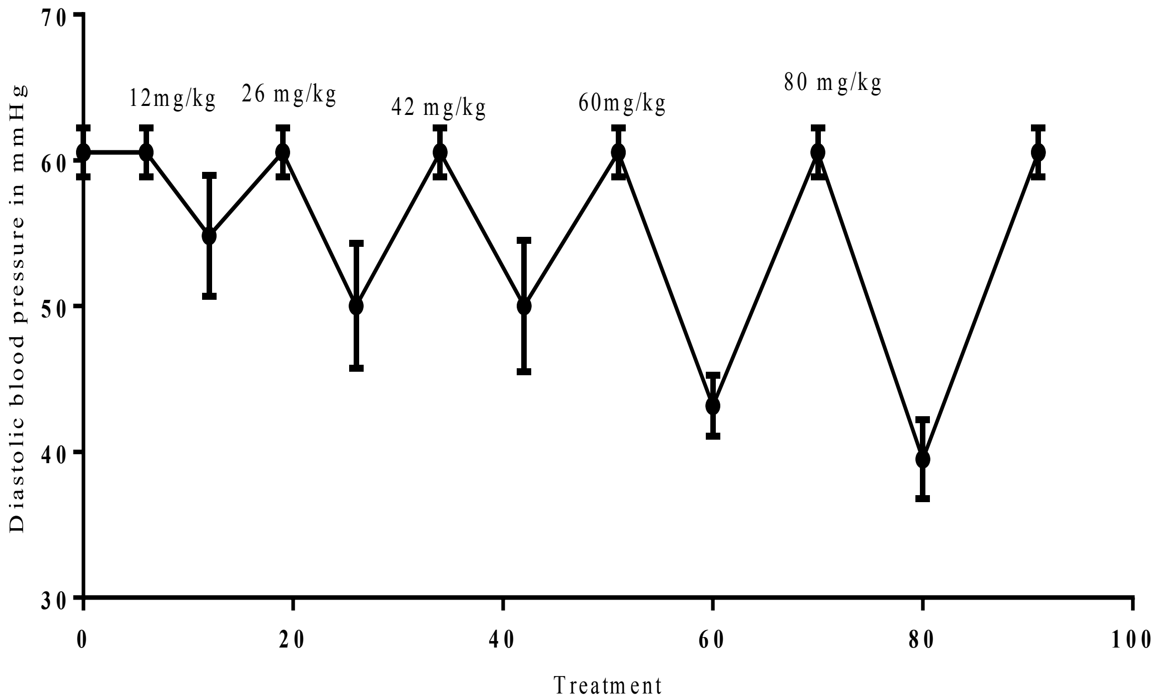


Figure 4-6: Effect of *Ruta chalpensis*on diastolic blood pressure in guinea pigs

A group of 6 guinea pigs was treated intravenously (via external jugular vein) with increasing doses (12, 26, 42, 60 and 80 mg/kg) of extract. Blood pressure was continuously monitored via a transducer connected to the carotid artery. After each treatment with the extract, blood pressure was allowed to return to baseline prior to treatment with the next dose of extract. Comparison was done with paired t-test and p <0.05 was considered significant.

The calculated pulse pressure (SBP-DBP) on guinea pigs before and after treatment with each dose of extracts (12, 26, 42, 60 and 80 mg/kg) was found to be unchanged at all doses. There were no significant differences (p=0.526) between pulse pressure before and after treatment of guinea pigs with 70% ethanol extract of the plant, suggesting that the aerial part of extract of *Ruta chalpensis* lowered systolic and diastolic blood pressure equally (Figure 4.7).

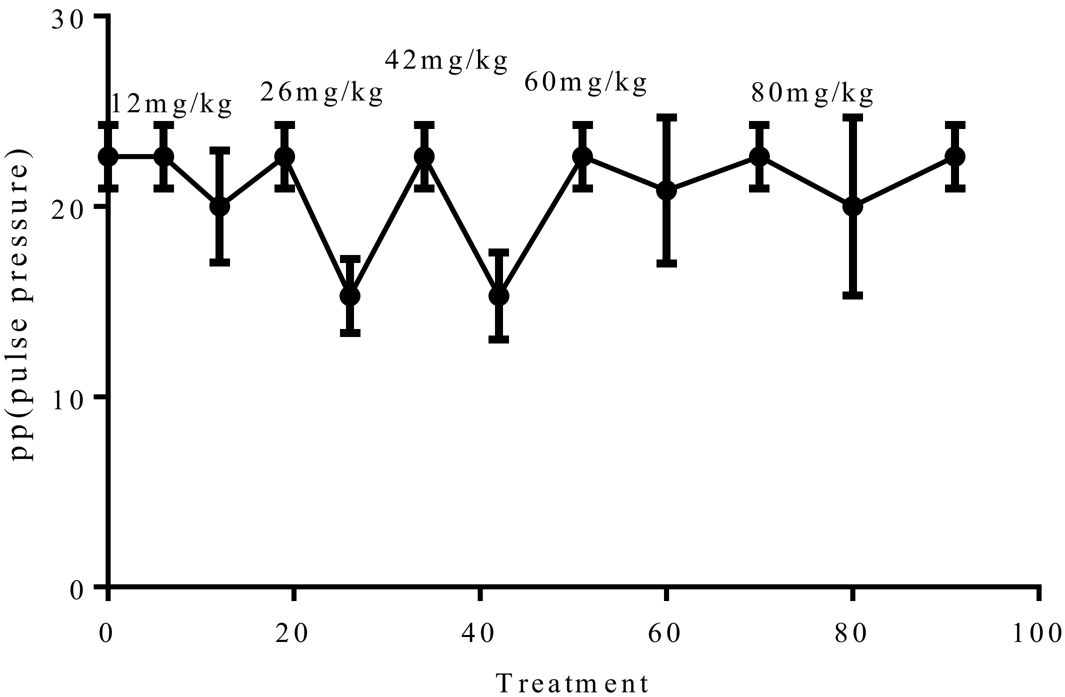


Figure 4-7: Effect of *Ruta chalpensis*on pulse pressure (SBP-DBP) in guinea pigs

Comparison was done with paired t-test and p <0.05 was considered significant

The calculated Mean Arterial Blood Pressure in guinea pigs is:

$$\text{DBP} + \frac{1}{3}(SBP - DBP)$$

Or DBP+1/3(PP)

The mean arterial blood pressure of guinea pigs before and after treatment with extract with doses of (12, 14, 16, 18 and 20) mg/kg was measured. Mean arterial blood pressure of the guinea pigs treated with extract with each dose of extract was significantly reduced (p<0.001) compared with guinea pigs before treatment, again in a dose dependent manner, with higher doses of extract causing an increasing lowering of mean arterial blood pressure (Figure 4.8).

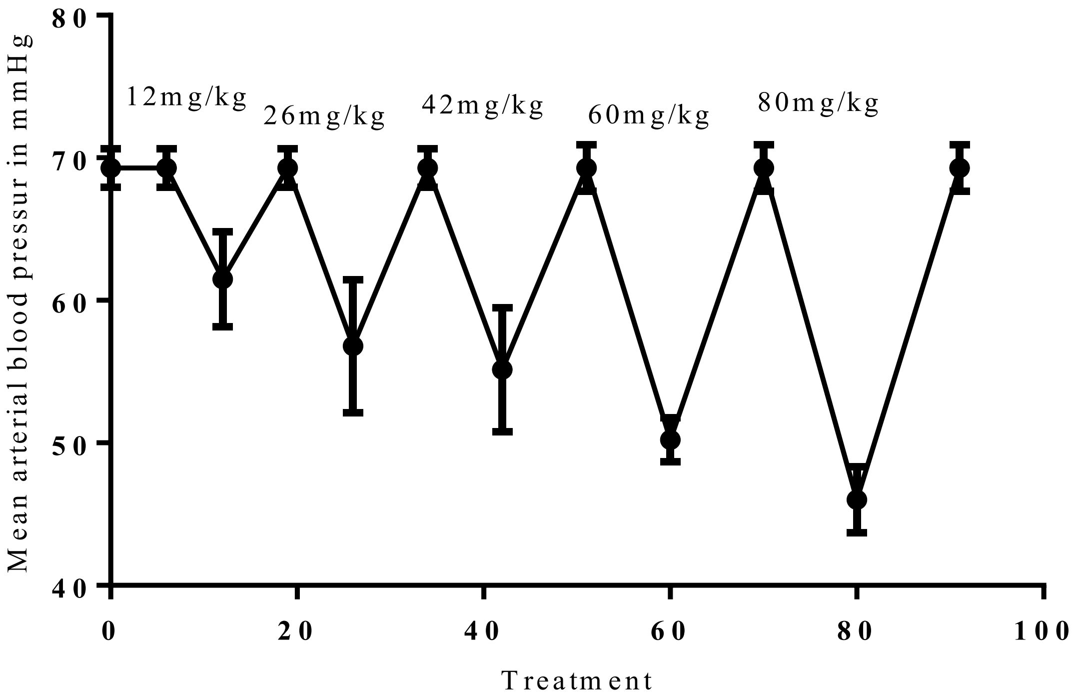


Figure 4-8 : Effect of *Ruta chalpensis*on mean arterial blood pressure in guinea pigs

Comparison was done with paired t-test and $p < 0.05$ was considered significant.

5. DISCUSSION

As described by Giles *et al*., 2009, hypertension is usually defined by the presence of a chronic elevation of systemic arterial pressure above a certain threshold value. According to journal of biological chemistry thematic mini-review series, 2010, nearly a quarter of the world's adult population is hypertensive. One community-based cross sectional study done in urban Addis Ababa showed that the age adjusted prevalence of high blood pressure was 31.5% among males and 28.9% among females. However, according to Tesfaye *et al*., 2009 only 35.2% of the hypertensive subjects were aware of their high blood pressure and only 11% were on treatment with target blood pressure attained is 25.6%.

According to Street and Prinsloo, 2013, A large number of people in both developing and developed countries rely on medicinal plants and medicinal plant products to maintain their health or treat illnesses, and as stated by Pugalendi *et al*., 2005, natural remedies for treating a wide variety of diseases have been investigated for centuries, Even people using only allopathic medicine throughout their lives are likely to be somewhat medicinal plant reliant as 20-25% of drugs prescribed are plant derived. As described by Shigeta *et al.,*2000, It is believed that about half of the top 25 best selling medicines in the world originate from natural materials, including plant substances. These all is because of its accessibility, low cost, and their multiple medicinal uses.

As reported by Shehadeh *et al*., 2007*, Ruta chalepensis,* is widely distributed in the Mediterranean and the Middle East region and The aerial part of this plant is used in traditional herbal medicine in many countries for treatment of wide variety of diseases. As reported by Araya *et al*., 2015, the leaf of *Ruta chalpensis* is used in northern part of Ethiopia for treating cough, flue, and malaria.

According to Geetha and Geetha, 2014, Phytochemical analysis is of best importance in identifying new source of therapeutically important compounds, and it is necessary for further extraction, purification and separation of the active medicinal compound.

In our qualitative chemical screening of *Rut chalepensis* 70% ethanol extract It was found positive for alkaloids, chromophores, polyphenols, saponins, phytosteroides, withanoids, flavonoids and tannins. Our finding was in line with study by Kacem *et al.,* 2015, which was done on phytochemicals and biological activities of *Ruta chalpensis* ,the presence of different secondary metabolites were assessed by using different solvent extracts, and it was found positive for phenolic compounds, flavonoids, tannins, alkaloids, comparison was done using different solvents and quantified, and the ethanol extract showed high amount of this phytochemicals. Though the present study uses one solvent system which is 70% ethanol and the phytochemicals were not quantified, the study showed the presence of these phytochemicals and it is in line with the above study.

And the results are consistent with the result found by Tukue and Babu, 2014, on phytochemical screening and antibacterial activity of two common terrestrial medicinal plants *Ruta chalpensis* and *Rumex nervosas,* which was reported positive for alkaloids, phenols, Tannins, flavonoids, sapponins and cardiac glycosides, where as negative for Antraquinone glycosides. But the present study was slightly positive for antraqunon glycoside and cardiac glycoside this might be because of polarity of the compound present in the plant, On biological factors, such as genotype, as well as environmental factors like, temperature, salinity, water, stress, light intensity and also the polarity of the solvent system contribute to the variation of the result. This result is also similar to the report by Fatma, 2011, which was done on medicinal plant from Jordan in the treatment of diabetes: traditional uses vs. *in vitro* and *in vivo* evaluations, the report revealed the presence of alkaloid, flavonoid, tannins, steroid/triterpins, rutin a flavonoid glycoside, which are responsible for its biological activity.

As described by Khlifi, *et al.,* 2013, DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolorizes the DPPH solution and the degree of colour change is proportional to the concentration and potency of the antioxidants. Antioxidant activity is then measured by the decrease in absorption at 517 nm, a large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test. As it was observed in table 4.2 the 70% ethanol extract of *Ruta chalpensis* showed antioxidant activity in dose dependent manner and it also showed a comparable result with the standard ascorbic acid.

The IC_{50} was computed to be 21.72mM of ascorbic acid and 58.20 mM of 70% ethanol extract of *Ruta chalpensis,* and an inhibition of 88.70% and 96.96% at 500ug/ mL of the extract and the standard ascorbic acid respectively, this is comparable with the result obtained from the study by Kacem, M. *et al.,* 2015, in the study the extract of *Ruta chalpensis,* ameliorates LPS- induced oxidative stress, through the elevation of antioxidative enzyme activities, such as catalase, supper oxide dismutase, and glutation peroxidase, this study discovered that the antioxidant enzymes were decreased by LPS treatment. However, the ethanol and the ethyl acetate extracts restored the activities of the ant oxidative enzymes, so, this study supports our result, and though the present study was done in Spectrophotometric analysis it also has antioxidant activity by the above stated possible mechanism.

As stated by Kasim,M. *et al.,* 2014, on protective effect of *Ruta chalpensis* L.extract on oxidative stress and liver-kidney function induced by polymicrobial sepsis in rats,this plant can control sepsis induced reactive oxygen species either by directly scavenging or by enhancing indogenious antioxidant defence system, and also the free radical scavenging (antioxidant) activity of the plant is due to the different secondary metabolites and they probably contribute to the effectiveness in therapeutic use, according to Pourmorad *et al.*, 2006; Nimmi 2012 the secondary metabolites like flavonoids are known with the antioxidant activity by the mechanism of scavenging and a phenolic compounds are mainly attributed to their redox properties, which allow them to act as reducing agent, hydrogen donor and quencher of singlet oxygen. These secondary metabolites present in the plant extract as our qualitative phytochemical screening method shows, might contribute to their antioxidant activity. From the above result the plant has a noticeable effect on the scavenging of free radicals this activity also increases with increasing concentration.

For *ex vivo* experiments, to achieve a baseline contraction (100%) of guinea pig aortas, the tissues were treated with high potassium chloride concentrations (K^+ =80mM). As it was suggested by Ghayur and Gilani, 2005, K^+ at high doses (>30mM) induced smooth muscle contractions through opening of voltage-dependent slow Ca^{2+} channels and as described by Moazedi, *et al*., 2010, K^+ is used as a device to avoid G protein coupled receptor stimulation and active smooth muscle by changing the calcium equilibrium potential and at some value above the resting level.

In our finding the mean percentage relaxation of guinea pigs aortas treated with 2 and 4 mg/mL of extract, was found to be 10.88 ± 2.87% and 27. 15 ±4.20 %, which was not significantly relaxed (p=0.807) for treated with 2 mg/mL and (p<0.94) for treated with 4 mg/mL as compared with the baseline contraction. However, treatment of isolated aortas with increasing doses of extract caused significant (p<0.001) relaxation of aortas as compared to the baseline contraction. These results show that 70% ethanol extract of aerial part of *Ruta chalpensis* resulted relaxation of aortic tissue dose dependently.

This may be possibly due to chemicals such as rutin, chalepesin and bergapten found in aerial parts of the plant. As reported by Heredia *et al.* 2011, that rutin and quercetin induce endothelium dependent vasorelaxation in rat thoracic aorta. These authors proposed that the vasodilator effect of quercetin might be mediated by guanylylcyclase- and cyclooxygenase-dependent pathways, while the vasodilatation by rutin might be via a nitric oxide guanylylcyclase pathway. Rutin flavonoid can be hydrolyzed to quercetin in the gastrointestinal tract. As stated by Beatriz *et al.*, 2010, the hydrolyzed rutin flavinoid (quercetin) causes smooth muscle relaxation by different mechanism such as stimulating nitric oxide production, bioavailability and activity, blocking calcium channel and inhibiting the endothelin -1 (ET-1) at gene transcription level.

As reported by Gross, 2004 on flavonoids and cardiovascular disease, rutin and its derivatives quercetin caused smooth muscle relaxation in rat aorta ring.

In the present study there were significant ($P < 0.001$) reductions in systolic, diastolic, and mean arterial blood pressure of normotensive guinea pigs after the treatment of extracts as compared to baseline blood pressure of guinea pigs. But the effect of *Ruta chalpensis* in pulse pressure of guinea pigs was not statistically significant (p=0. 526) at lower doses of the extracts. This reduction of blood pressure may be possibly due to reduction in oxidative stress, interference of RAAS, and /or improvement of endothelial cells by phytochemicals present in the aerial part of *Ruta chalpensis.*

Systolic and diastolic blood pressure was significantly reduced at all doses of the extracts, which shows the extract has dose dependent blood pressure lowering effect. At extract dose of 12 mg/kg, systolic blood pressure was reduced (84.54± 0.71 to 65.33± 1.92) mmHg and, higher doses of *Ruta chalpensis* extract 80 mg/kg causes increasingly higher reduction in systolic blood pressure (84.54 ± 0.71 to 58.83 ± 3.87) mmHg. This could be possibly due the presence of major compounds, rutin, chalpensin, bergapten and hydrolyzed rutin (quercitin) are also responsible for the blood pressure lowering effect.

As described by Beatriz *et al*., 2010, the effect of *Ruta chalpensis* 70% ethanol extract showed the above effect presumably by the secondary metabolites like, alkaloids, flavnoids, coumarins, and phenols detected in the qualitative phytochemical screening of the plant extract, the above mentioned compounds are responsible for a large variety of pharmacological actions which include stimulation of endothelial nitric oxide synthesis and inhibition of angiotensin converting enzyme as described by this author *Ruta chalpensis* is also a reach source of important secondary metabolites such as furanocoumarins and alkaloids.

As revealed by Ugusman *et al.,* 2014 on role of rutin on nitric oxide synthesis(eNOS) in human umbilical vein endothelial cells, rutin caused up regulation of endothelial nitric oxide synthase mRNA expression and increase in endothelial nitric oxide synthase protein level and endothelial nitric oxide synthase activity. The increase in endothelial nitric oxide synthase mRNA expression caused more endothelial nitric oxide synthase protein to be synthesized. The higher amount of endothelial nitric oxide synthase protein led to a higher level of endothelial nitric oxide synthase activity that results increased production of nitric oxide. As a result there is vasodilatation of smooth muscle which leads to reduction in blood pressure.

Another study conducted by Larson *et al.,* 2010 on a review of efficacy and mechanisms quercetin, improve endothelial by down regulating the endothelin-1(ET-1) at gene level and this causes increasing of production, bioavailability and activity of nitric oxide that results to relaxation of smooth muscles and this leads to decrease in blood pressure.
As described by Cai and Harrison, 2000, increased reactive oxygen species levels inactivate the vasodilator nitrogen oxide leading to endothelial dysfunction and vasoconstriction which results hypertension. since quercetin has free radical attacking ability and improving nitric oxide production that leads to vasodilatation of smooth muscles. As a result it might possibly reduce blood pressure.

As stated by Boots *et al.,* 2011, in human peripheral blood mononuclear cells, quercetin, scavenging reactive oxygen species by not only offer protection against oxidative stress, but also simultaneously mitigate inflammation by inhibiting tumor necrosis factor α(TNFα)production as well as tumor necrosis factor α gene expression via modulation of NF-κB. Another study by Perez-Vizcaino *et al*., 2009, explained that quercetin increased the bioavailability and the biological activity of nitric oxide via either a direct superoxide anion scavenger effect or by inhibition of superoxide generating enzymes. Another possible mechanism of blood pressure reduction as stated by Larson *et al.,* 2010, on the effect of a single dose of quercetin on cardiovascular function in normotensive and hypertensive men, quercetin might reduce oxidative stress, interfere with the (RAS), and /or improve endothelial and,/ or vascular function.

6. CONCLUSION

The study shows, the aerial part of the 70% ethanol extract of *Ruta chalpensis* is reach in secondary metabolites like phenols, saponins, phytosteroids and withanoids, flavonoids, tannins, and alkaloids.

The extract also shows in vitro antioxidant activity using the DPPH method, and the intravenous administration of the areal part 70% ethanol extract of *Ruta chalpensis* exhibited blood pressure lowering effect in normotensive anesthetized guinea pigs in dose dependent manner.

In *ex vivo* tissue preparation, the areal part70% ethanol extract of *Ruta chalpensis* exhibited inhibited effect on high K^+ induced contraction of isolated aorta in *ex vivo* tissue preparation, so it can be concluded, the present findings provide scientific justification for the traditional use of the plant as antihypertensive agent in different countries.

7. RECOMMENDATIONS

From this study it is recommended that;

- To identify the chemical compound which is responsible for the observed medicinal activity further fractionation and isolation of active compound is required.

- This study focused on the hypotensive effect of the plant extract on normotensive guinea pigs, further work is required on experimentally induced hypertensive animal models.

- Further work is recommended on effective dose and lethal dose of the extract.

- The safety of the plant material should be done in future.

- Other parts of this plant should be done comparatively to that of the areal part in order to identify best part of this plant in treatment of hypertension

- The exact mechanism of action of the plant material to reduce blood pressure should be done.

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