Evaluation of the diuretic activity of aqueous and 80% methanol extracts of *Foeniculum vulgare* Mill (Apiaceae*)* leaf in rats

By

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This is to certify that the thesis prepared by Abdurazak Jemal entitled "Evaluation of the diuretic activity of aqueous and 80% methanol extracts of *Foeniculum vulgare* leaf in rats" and submitted in partial fulfillment of the requirements for the degree of Master of Sceince in Pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Evaluation of the diuretic activity of aqueous and 80% methanol extracts of *Foeniculum vulgare* Mill. (Apiaceae) leaf in rats

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Addis Ababa University, 2015

The leavf of *Foeniculum vulgare* is used in Ethiopian traditional medicines as a diuretic agent for the treatment of urine retention and hypertension. As this claim has not been investigated scientifically, the aim of this study was to evaluate the diuretic effect of the aqueous and 80% methanol leaf extracts of *Foeniculum vulgare* in rats. Spargue Daweley rats were divided into eight groups each comprising six rats. Control group received distilled water 10 mL/kg, the reference group received hydrochlorthiazide 10 mg/kg and the test groups were administered different doses of aqueous or 80% methanol extract (100, 200 and 400 mg/kg) orally. Urine output was collected up to 24 h and analyzed for electrolyte contents. Rats treated with 200 and 400 mg/kg doses of aqueous and 80% methanol extract of *Foeniculum vulgare* displayed an increased urine volume ($p<0.001$). However, 100 mg/kg dose of both extracts failed to produce significant increase in 24 h urine volume compared to control groups. Both extracts increased natriuresis, kaliuresis and chloriuresis ($p<0.001$) at the middle and higher doses. The result indicated that the plant is endowed with significant activity, aqueous being better than 80% methanol extract, providing proof for the traditional claim. The major constituents like flavonoids, tannins, terpenoids and alkaloids found in the plant might have contributed to the observed diuretic activity.

Keywords: *Foeniculum vulgare*, Diuretic Activity, Natriuresis, Kaliuresis

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List of Abbreviations

ADH	Antidiuretic Hormone
ANOVA	One-Way Analysis of Variance
CD	Collecting Duct
CHF	Congestive Heart Failure
CNS	Central Nervous System
DCT	Distal Convoluted Tubule
GFR	Glomerular Filtration Rate
ISE	Ion Selective Electrode
OECD	Organization of Economic Co-operation and Development
PCT	Proximal Convoluted Tubule
S.E.M	Standard Error of Mean
UT-A	Urea Transporter-A
UT-B	Urea Transporter-B
V2R	Vasopressin Receptor 2

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1. Introduction

1.1. History of Diuretics

The term diuretic is derived from the Greek ‘diouretikos’ which means ‘prompting urine’. By definition, diuretics are chemicals that increase the rate of urine formation (Suhas et al., 2012). In strict sense, a diuretic is an agent that increase urine volume, while a natriuretic is substance that increase in renal sodium excretion. Because natriuresis usualy increases water excretion, they are called diuretics. The term aquaretic is applied to drugs that increases excretion of solute free water differentiating these drugs from traditional diuretics, which enhaced solute and water excretion. By increasing urine flow rate, diuretic usage leads to increased excretion of sodium and water in edematous conditions (Suresh et al., 2010).

The history of diuretics goes back to an ancient time (Das et al., 2014). Paracelsus discovered the use of mercurous chloride (calomel) as a diuretic for treatment of edema in 1553. The diuretic effect of organic mercurial was discovered by accident when they were being used to treat syphilis in 1919. The idea of diuretic therapy for hypertension originated in 1937 with the discovery that sulfonamide was found to cause metabolic acidosis and mild diuresis in patients. In 1957, chlorothiazide was isolated during a search for more potent inhibitors of carbonic anhydrase and found to be a more effective diuretic of that time (Marvin et al., 2009). The search for better classes of diuretics based on the structure of chlorothiazide and sulfonamide derivatives resulted in the development of furosemide and ethacrynic acid. Although these compounds were proved to be very effective in promoting sodium excretion, they caused potassium loss as unwanted effects. The the search for potassium sparing diuretics resulted in the introduction of spironolactone, amiloride and triameterene (George, 2011).

Drug induced diuresis is valuable in many life threatening conditions such as congestive heart failure, kidney disease, hypertension, acute edema of the lung and ascites. Although most of the diuretics proved to be very effective in promoting sodium excretion**,** all cause potassium loss and this prompted a search for new diuretic agents that retain therapeutic efficacy and yet devoid of potassium loss (Eswaraiah et al., 2012; Nayak et al. 2013). Currently there is growing interest in research of new herbal diuretics due to side effects associated with synthetic diuretic agents.

1.2. **Renal Anatomy and Physiology**

The kidneys are the main organs of homeostasis that maintain the acid-base and water salt balance of the blood. The primary function of the kidneys is to maintain a stable internal environment for optimal cell and tissue metabolism by separating urea, mineral salts, toxins, and other waste products from the blood. The kidneys also conserve water, salts, and electrolytes. At least one kidney must function properly for life to be maintained (Marieb et al., 2007).

The kidneys lie against the posterior abdominal wall at the level of vertebrae thoracic 12 (T12) to lumber 3 (L3). The right kidney is slightly lower than the left because of the space occupied by the liver above it. Each kidney weighs about 160 g and measures about 12 cm long, 5 cm wide, and 2.5 cm thick about the size of a clenched fist. The lateral surface is convex while the medial surface is concave and has a slit, the hilum**,** where it receives the renal nerves, blood vessels, lymphatic vessels, and ureter. Under normal resting conditions, the large renal arteries deliver one fourth of the total cardiac output, about 1200 mL, to the kidneys each minute, which attests to their importance in controlling blood volume and composition (Saladin., 2003).

The kidney is protected in the body by three layers of connective tissues: (i) fibrous renal fascia, immediately deep to the parietal peritoneum, which binds the kidney and associated organs to the abdominal wall; (ii) the adipose capsule, a layer of fat that cushions the kidney and holds it in place; and (iii) the renal capsule, a fibrous sac that anchored at the hilum and encloses the rest of the kidney and protects the kidney from trauma and infection. Collagen fibers extend from the renal capsule, through the fat, to the renal fascia. The renal fascia is fused with the peritoneum on one side and the deep fascia of the lumbar muscles on the other. Thus the kidneys are suspended in place by network of collagen fibers (Saladin., 2008).

The renal parenchyma, the glandular tissue that forms the urine is divided into two zones: an outer renal cortex and an inner renal medulla. Extensions of the cortex called renal columns project toward the sinus and divide the medulla into 6 to 10 renal pyramids. Each pyramid is conical, with a broad base facing the cortex and a blunt point called the renal papilla facing the sinus. The papilla of each renal pyramid is cushioned in a cup called a minor calyx, which collects its urine. As shown in Figure 1, two or three minor calices converge to form a major calyx**,** and two or three major calices converge to form the renal pelvis. The ureter is a tubular continuation of the renal pelvis that drains the urine down to the urinary bladder (Saladin, 2003).

Nephrons are the structural and functional units of the kidneys. Each kidney contains over one million which carry out the processes that form urine. A nephron consists of two principal parts: a renal corpuscle where the blood plasma is filtered and a long renal tubule that processes this filtrate into urine. The renal corpuscle consists of a ball of capillaries called a glomerulus, enclosed in a two layered glomerula capsule. The fluid that filters from the glomerular capillaries collects in the capsular space between parietal and visceral layers and flows into the renal tubule

on one side of the capsule. The renal tubule is a duct that leads away from the glomerular capsule and ends at the tip of a medullary pyramid. It is divided into four major regions: the proximal convoluted tubule, loop of Henle, distal convoluted tubule and collecting duct. Each region of the renal tubule has unique properties and roles in the production of urine (Rang et al., 2007).

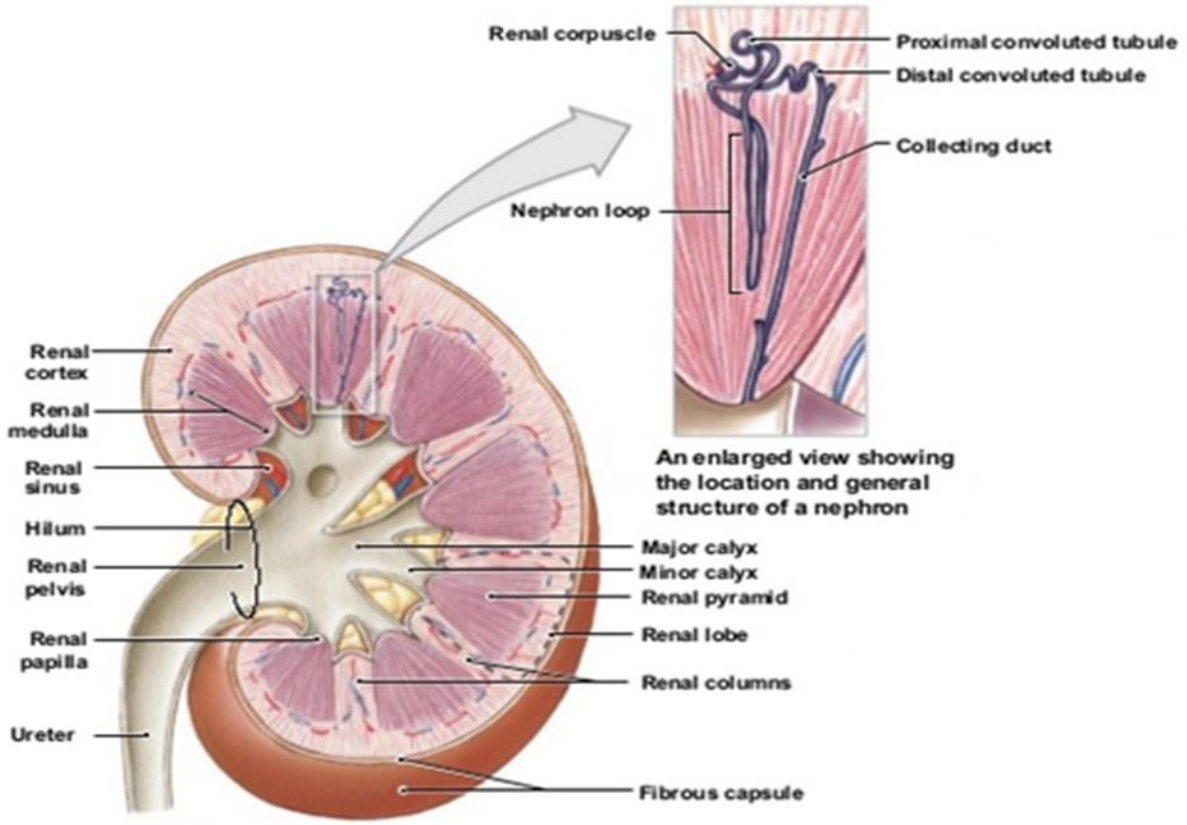

Figure 1: Major anatomical features of the kidney and nephron (Saladin., 2008)

The proximal convoluted tubule (PCT) arises from the glomerular capsule is the longest and most coiled of the four regions of renal tubule has epithelium with prominent microvilli, which attests to the great deal of absorption that occurs here. After coiling extensively near the renal corpuscle, the PCT straightens out and forms a long U shaped nephron loop. The first portion of the loop, the descending limb, passes from the cortex into the medulla. At its deep end it turns 180° and forms an ascending limb that returns to the cortex (Saladin, 2003).

The nephron loop is divided into thick and thin segments. The cells in thick loop of Henle are heavily engaged in active transport of salts, so they have very high metabolic activity and are loaded with mitochondria. The thin segment forms the lower part of the descending limb has low metabolic activity, but is very permeable to water (Barrett et al. 2010). When the nephron loop returns to the cortex, it coils again and forms the distal convoluted tubule (DCT), which is the shorter and less convoluted end of the nephron. The DCT of several nephrons drain into a straight tubule called the collecting duct (CD), which passes down into the medulla. Several collecting ducts merge to form a larger papillary duct near the papilla; about 30 of these drains from each papilla into its minor calyx (Saladin., 2008).

1.3. **Conventional Diuretics**

1.3.1. **Mechanism and Site of Actions**

Conventional diuretics are categorized into different classes based on their distinct mechanism and site of actions. Diuretics suppress sodium and water reabsorption by inhibiting the function of specific proteins that are responsible for the transportation of electrolytes across the epithelial membrane. Osmotic diuretics inhibit water and sodium reabsorption by increasing intratubular osmotic pressure. Figure 2 shows major site of diuretic action in renal tubule (Michael., 2009).

The proximal tubule reabsorbs 65-70% of filtered salt, 60% filtered water and the major site for sodium bicarbonate reabsorption (85%), which requires sodium-proton exchanger (antiporter) and carbonic anhydrase enzyme. Carbonic anhydrase inhibitors exert diuretic effect by inhibiting sodium bicarbonate transport. The PCT is the major site for the secretion of organic acid and bases to the tubular lumen; this is also the mechanism by which most diuretics reach thier sites of action (Ives., 2009).

The thick ascending limb (TAL) of loop of Henle actively reabsorbs sodium and potassium chloride via the Na^+-K^+-$2Cl^-$ cotransporter that is responsible for 25% of salt reabsorption is impermeable to water and thus a urine diluting segment of th tubule. Active sodium reabsorption at TAL contributes to the hypertonicity in medullary interstitium. Loop diuretics act on the TAL of the loop of Henle, inhibiting the transport of sodium chloride out of the tubule into the interstitial tissue by inhibiting the co-transporter on the apical membrane site (Smith., 2014).

The DCT reabsorbs about 5% of NaCl via sodium chloride co-transporter (Na^+/Cl^- symporter). Thiazide diuretics inhibit the function of this symporter and reduce sodium reabsorption at DCT. The CD is the final site for 1-2% of sodium chloride reabsorption. Sodium reabsorption in the collecting tubule system is mediated by a sodium channel and regulated by aldosterone hormone (Jackson., 2006). Aldosterone antagonists exert diuretic effect by inhibiting aldosterone receptor. The CD is the major site for potassium secretion which is driven by a negative lumen potential established by sodium reabsorption. Diuretics that act on upstream segments of the renal tubule increase sodium concentration in the tubular fluid, resulting in increased sodium absorption in the collecting duct systems causing hypokalemia by increasing potassium secretion. Potassium sparing diuretics inhibit Na^+ reabsorption in the collecting duct systems and thus reduce K^+ secretion (Adedoyin et al., 2012).

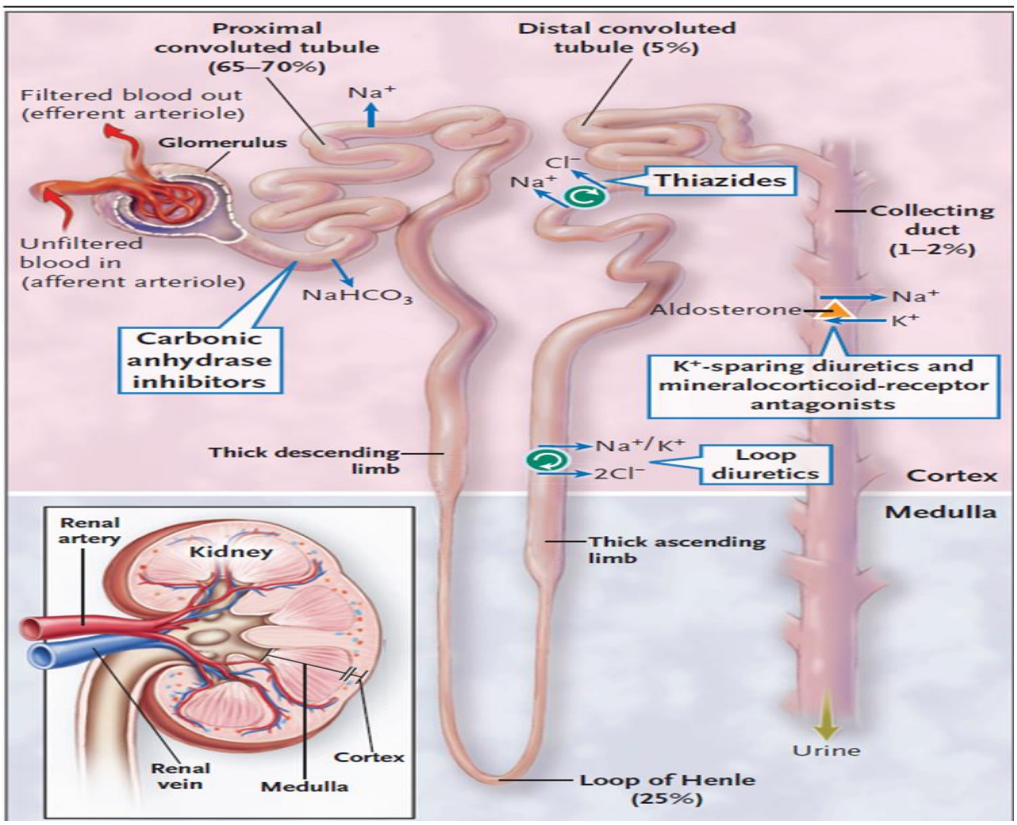

Figure 2**:** Tubule transport systems and sites of action of diuretics (Michael et al., 2009)

1.3.2. **Therapeutic Importance**

Major purpose of diuretic therapy is to decrease body fluid volume and adjust electrolyte balance in the management of pathological conditions such as edema in congestive heart failure, certain renal diseases and hypertension (Dubey et al., 2010). Thiazide diuretics may be used either alone or in combination with other pharmacotherapy for the treatment of hypertension. Loop diuretics can provide immediate diuresis and are used for heart failure and in lieu of thiazides in patients with compromised renal function. Potassium-sparing diuretics provide added benefit to other pharmacotherapy in patients with primary hyperaldosteronism, heart failure, or post-acute myocardial infarction. Carbonic anhydrase inhibitors have limited use for diuresis; however, they are used to reduce intraocular pressure and treat acute mountain sickness (Clarke et al., 2001).

1.3.3. **Adverse Effects**

The common side effect associated with most diuretics is the distortion of water and electrolyte balance such as hypokalemia, acid-base imbalance, and hyponatremia (Khan et al, 2012). Most diuretics increase elimination of potassium, which when left uncorrected can increase the risk of serious heart rhythm disturbances. A potential side-effects associated with a potassium-sparing diuretic is a high level of potassium in people who already have a high potassium level or in those who have kidney disease. Carbonic anhydrase inhibitors cause electrolyte imbalance like hyperchloremic metabolic acidosis (due to increased bicarbonate excretion and inhibition of titratable acid and ammonia secretion) and allergic reactions to sulfonamides. Expansion of extracellular fluid volume by osmotic diuretics may result in hyponatremia causing central nerve system symptoms like nausea, headache, and vomiting. Loop and thiazide diuretics cause hypokalemic metabolic alkalosis (because they cause potassium and acid secretion), extracellular volume depletion, hypotension, and allergic reactions (Jentzer et al., 2010, Mitra et al., 2012).

1.4. **Novel Diuretics**

Diuretics relieve clinical symptoms and signs in patients with edematous conditions. However, long-term use of conventional diuretics can have several adverse effects including diuretic-induced potassium imbalances that diminish the efficacy and safety of conventional diuretics. Therefore, inventing a new diuretic that doesnot cause electrolyte disturbance becomes essential and various new diuretics are on the way of development. Adenosine A_1 receptor antagonists, vaptans and urea transporter inhibitors are the new classes of diuretics on clinical and clinical trial phases of development (Givertz et al., 2007, Mullera et al., 2011).

1.4.1. **Adenosine A_1 Receptor Antagonists**

Adenosine is an adenine nucleoside that is produced by the hydrolysis of adenosine triphosphate (ATP). The paracrine nature action of adenosine is mediated by receptors that activate secondary messenger systems resulting in hemodynamic effects in the vascular beds of the kidney, brain and heart. Adenosine initiates its biological effects via the four receptor subtypes, namely the A_1, A_{2A}, A_{2B} and A_3 all of which are G-protein-coupled receptors. The A_1 and A_{2A} receptors have high affinity while the A_{2B} and A_3 receptors show relatively lower affinity for adenosine. A_1 and A_3 are coupled to G_i proteins and inhibit adenylyl cyclase, while A_{2A} and A_{2B} receptors activate G_s protein to stimulate adenylyl cyclase (Sheth et al., 2014). In the heart and other vascular beds, adenosine function as a vasodialator through activation of A_{2A} receptors. In the kidney, however, activation of A_1 receptors in the afferent arteriol results in vasoconstriction that reduces renal blood flow and glomerular filtration rate (GFR). In addition, adenosine has direct luminal effects, enhancing sodium reabsorption in the PCT (Vallon et al., 2008).

Inhibition of sodium reabsorption in the PCT would be beneficial in diseases associated with volume retention. However, the resulting increase in delivery of sodium and chloride to the TAL loop of Henel activates tubuloglomerular feedback, which causes a decrease in GFR. Induction of diuresis and natriuresis without causing the compensatory decrease in renal function would be beneficial in diuretic resistant patients with volume over load. Therefore, effective inhibition of sodium reabsorption without reducing renal function is desirable and blockade of adenosine A_1 receptors may provide such novel therapy. In addition, A_1 receptor antagonists may have a role in the treatment of ischemic injury to the kidney by maintaining afferent arteriole vasodilatation preserving the GFR (Schnackenberg et al., 2003, Welch, 2015).

Preclinical and clinical studies done in animals and humans demonstrated that the A_1 receptor antagonists enhance sodium excretion, increase GFR and attenuate the decline in glomerular filtration induced by loop diuretics (Jackson., 2002). KW- 3902 (rolofyline) is a novel, specific adenosine A_1 receptor antagonist shown in animal models to cause diuresis, in large part by inhibiting sodium reabsorption at tubular site (Givertz et al., 2007). It also significantly increased GFR and renal plasma flow in patients with congestive heart failure (CHF) in phase II trials (Voors et al., 2011). Other selective A_1 receptor antagonists in clinical trials includes BG-9719 (naxifylline) (Welch et al., 2015) and FK838 in clinical phase II (Baraldi et al., 2008) may emerge as a new class of renal friendly diuretics.

1.4.2. **Vasopressin Receptor Antagonists**

Anti-diretic hormone (ADH), also known as vasopressin, plays a central role in regulating body fluid homeostasis, serum osmolality, and vascular tone. The biological effects of vasopressin are mediated by three receptor subtype, all of which are members of the G-protien linked receptor family; V1a, V1b (V3) and V2 (Lehrich et al., 2013). When release is stimulated, the circulating vasopressin binds to the vasopressin receptors 2 (V2R) on the principal cells of CD stimulates insertion of water channels on the apical membrane increasing reabsorption of water, thereby producing an antidiuresis. As a consequence of receptor inactivation, the synthesis and transport of aquaporin-2 water channel proteins into the apical membrane of the CD cells is inhibited and this prevents free water reabsorption and causes increased urine volume. The increased diuresis produced by vasopressin receptor antagonists is quantitatively similar to diuretics such as furosemide, but it is qualitatively different because there is not a significant increase in the excretion of urine solutes (electrolytes) such as sodium and potassium (Narayen et al., 2012).

The aquaretic effect is, therefore, the hallmark of these compounds and distinguishes them from conventional diuretics. Vasopressin receptor antagonists are classified into two classes based on receptor selectivety as selective V2R antagonists (mozavaptan, lixivaptan, satavaptan, tolvaptan) and nonselective receptor antagonists (conivaptan) (Jagadeesh et al., 2014, Beg et al.,., 2015).

1.4.3. **Urea Transporter Inhibitors**

Urea transporters are a family of proteins that facilitate the passive transport of urea across the plasma membrane in descending limb of loops of Henle and inner medullary collecting duct. Intrarenal urea recycling is involved in the urinary concentrating mechanism by two types of urea transporters: urea transporter- B (UT-B) and members of urea transporter-A (UT-A). UT-A subfamily includes six members (UT-A1-A6). UT-B can function as a water channel, in addition to its role as a urea transporter. UT-A1/A3 play important role in urine concentrating mechanism in the inner medullary collecting duct (Aditya et al., 2012; Yang., 2015).

Urea transporter inhibitors have a different mechanism-of-action from conventional diuretics, which block salt transport across kidney tubule epithelial cells. Urea transporter inhibitors can be widely used to increase renal water excretion in conditions associated with total body fluid overload, including congestive heart failure, ascites and nephrotic syndrome. By disrupting countercurrent mechanisms and intrarenal urea recycling, urea transporter inhibitors, alone or in combination with conventional diuretics, may induce a diuresis in states of refractory edema where conventional diuretics are ineffective. PU-48 evaluated in rat models shows that urea transporter inhibitors may be developed as a novel class of diuretics performing urea-selective diuresis without disturbing electrolyte excretion and metabolism (Yang., 2015).

1.5. **Botanical Diuretics**

Medicinal plants have been known for millennia and are thought as a rich source of therapeutic agents for the prevention of diseases (Snigdha et al., 2013). Traditional medicines are popular in developing countries and most of the population relies on folk remedies for their primary health care needs (Reddy et al., 2011). The reason for this may be that some plants show effects comparable to the outcome obtained from western medicines with lesser side effects (Gupta et al., 2011). One of the application areas of botanicals is their diuretic effect. This is supported by a number of researches published that evaluated the degree of clinical support for the use of folklore medicines as a diuretic. Such evidence is needed in order to determine if there is any scientific basis for their use. Many investigators demonstrated that studies of herbals in folklore use as diuretics were in progressive evaluation and might be valuable tools used in human disease (Kumar et al., 2010, Jaysree et al., 2011, Praveen et al., 2013).

There are several plants species reported to have promising diuretic effects including: *Olea europaea* (Somova et al., 2003) *Carissa edulis* (Nedi et al., 2004), *Rumex abyssinicus* Jack (Mekonnen et al., 2010), *Ruta graveolens* L (Jayakody et al., 2011), *Ajuga remota* Benth (Hailu et al., 2014), *Costus speciosusin* (Prabhu et al., 2014), *Buchanania angustifolia* Roxb., and *Buchanania lanzan* Spreng (Hullatti et al., 2014), *Flueggea leucopyrus* (Ellepola et al., 2015), *Cissampelos pareira* (Sayana et al., 2014), and *Nigella sativa* (Asif et al., 2015).

1.6. **The Experimental Plant *(Foeniculum vulgare)***

The Apiaceae is a large plant family consisting of approximately 200 genera and over 2,900 species grown world wide. Its representative vegetables are carrots, parsleys, celerys, as well as well-known spice plants such as fennels, anise, caraway, dill, and coriander (Bulajic et al., 2009). The family Apiaceace is one of the most numerous families within vegetable crops. This family is rich in secondary metabolites and represents numerous genera of high economic and medicinal value including essential oils (Olle et al. 2010).

Foeniculum vulgare M. (Figure 3) is a biennial medicinal and aromatic plant belonging to the family Apiaceae. Fennel is a perennial umbelliferous herb with feathery leaf. It grows to a height of up to 2.5 m with hollow stems. The leaf grows up to 40 cm long and the flowers are produced in terminal compound umbels. The fruit is a dry seed up to 10 mm in length (Moghtader., 2013). Fennel is generally considered indigenous to the shores of Mediterranean Sea, but has become widely domesticated in many parts of the world especially on dry soils near the sea coast and on the river banks (Rather et al., 2012).

Figure 3: Photograph of *Foeniculum vulgare*

Foeniculum vulgare M. known as fennel in English, ensilal in Amharic has long been considered as a medicinal and spice herb. Furthermore, it is a high value medicinal crop used as a diuretic, carminative, sedative, expectorant and antispasmodic agent (Kishore et al., 2012;). Fennel fruits are used to treat diseases like cholera, bile disturbances, nervous disorder, constipation and diarrhea (Elizabeth et al., 2014). Antioxidant and antimicrobial activity of fennel has also been reported (Sharafzadeh et al., 2011; Khan et al., 2014). In Ethiopia the leaf of *Foeniculum vulgare* is boiled in water and the decoction is taken orally as a diuretic agent (Suleman et al., 2012). The essential constituents of fennels fruits' are volatile oils. Transanethole, estragole, fenchone and limonene are the major constituents of the essential oil of fennel fruits. Phytochemical analysis of *Foeniculum vulgare* was reported to have alkaloids, glycosides, phenols, tannins, phytosterols, flavonoids and saponins (Chatterjee et al., 2012; Ouariachi et al., 2014).

1.7. **Rationale for the Study**

Numerous clinically used medicines are derived directly or indirectly from plant sources. While a good number of purified plant constituents have been developed as modern medicines, a vast majority of world population still uses herbal medicines for primary health care purpose. Herbs are effective in the treatment or prevention of various chronic diseases, such as diabetes melitus, ascites, cardiovascular disorders and renal diseases (Das et al., 2014). Diuretics are one class of medicines that used in the management of renal and cardiovascular diseases. The two widely used diuretics, thiazides and high ceiling loop diuretics have been associated with a number of adverse effects on quality of life (Mitra et al., 2012) which justifies the search for new better diuretics. Hence, this study is intended to evaluate the diuretic activity of aqueous and 80% methanol leaf extract of *Foeniculum vulgare* in rats.

2. Objective of the Study

2.1. General Objective

The main purpose of the study was to evaluate the diuretic activity of aqueous and 80% methanol leaf extracts of *Foeniculum vulgare* in rats.

2.2. Specific Objectives

- To evaluate the effect of aqueous and 80% methanol leaf extracts of *Foeniculum vulgare* on urine volume
- To determine the effect of aqueous and 80% methanol leaf extracts of *Foeniculum vulgare* on electrolyte concentration
- To determine effect on pH of the urine following administration of the leaf extracts.
- To assess acute toxicity profile of the plant

3. Materials and Methods

3.1. Drugs and Chemicals

The following chemicals and drugs were used in the experimental study: distilled water, normal saline (EPHARM, Addis Ababa, Ethiopia), absolute methanol (CARLO ERBA, Spain), and the standard drug hydrochlorothiazide 25 mg (Remedica Ltd., Cyprus- EU).

3.2. Experimental Animals

Healthy Spargue Daweley rats of either sex having a weight range of 180 – 200 g inbred in the animal house of the School of Pharmacy, Addis Ababa University were used for the experiment. The animals were housed in polypropylene cages (6 rats per cage) under standard environmental conditions and 12 h/12 h light/dark cycle. The animals were allowed free access to tap water and laboratory pellet. Each rat was placed in an individual metabolic cage (metabolic cage for rats, TECHNIPLAST, Italy) 24 h prior to commencement of the experiment for adaptation. The care and handling of animals were in accordance with internationally accepted OECD-425 (2008) guidelines for use of animals and the procedure was approved by the School of Pharmacy Ethics Committee, Addis Ababa University.

3.3. Plant Collection

The leaves of *Foeniculum vulgare* were collected in November 2014 from Assela town, about 175 km Southeast of Addis Ababa. Taxonomic identification was made by a taxonomist and a voucher specimen (AJ/001) was deposited at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University for future reference.

3.4. **Extraction of the Plant**

The collected leaves of *Foeniculum vulgare* were dried under shade at room temperature for a period of 2 weeks. The dried leaves were crushed to reduce the size using mortar and pestle. The dried and crushed leaves were then powdered finely and subjected to extraction.

3.4.1. **Aqueous Extraction**

One hundered fifty gram of the powdered leaves of *Foeniculum vulgare* was boiled at 100°C in about 800 mL of distilled water for thirty minute to simulate traditional uses, and cooled to room temperature for fifteen minutes. The decoction obtained was filtered and frozen at −20 ^{o}C and lyophilized using a lyophilizer (Lyophilizer, OPR-FDU-5012, Korea) until dried. The dried aqueous extract was collected and the yield was found to be 13% (w/w). The dried plant extract was reconstituted with distilled water for oral administration.

3.4.2. **Methanol Extraction**

One hundred fifty gram of the powdered leaves of *Foeniculum vulgare* was macerated with about 600 mL of 80% methanol for 24 h. The extract was filtered using Watman No-1 filter paper and the marc was remacerated twice using the same volume of solvent to exhaustively extract the plant material for a total of seven days. The menustrum was removed from the extract by evaporating under reduced pressure using rota vapour apparatus (BUCHI Rota vapour R-200, Switzerland) at 40 ^{o}C. The extract obtained was filtered and frozen at −20 ^{o}C and lyophilized until dried. The yield of the dry extract was found to be 12% (w/w).

3.5. **Acute Toxicity Study**

Five female rats were received orally the same doses (2000 mg/kg) of the aqueous and/or 80% methanol extracts. The animals were observed for obvious toxicities like diarrhea, weight loss, and paralysis for the first 24 h and for 14 days for any lethality (OECD-425., 2008).

3.6. **Grouping and Dosing of Animals**

Animals were randomly assigned into eight groups each consisting of six rats for diuretic test. The negative controls were treated with the vehicles used for reconstitutions (10 mL/kg of body weight, CONT). The positive controls were treated with hydrochlorothiazide 10 mg/kg (HCT10). Three treatment groups in each test were treated with different doses of the extract as follows: the aqueous extract with doses of 100 mg/kg (A100), 200 mg/kg (A200), and 400 mg/kg (A400); and 80% methanol extract with doses of 100 mg/kg (M100), 200 mg/kg (M200), and 400 mg/kg (M400). Dose selections was made based on the acute oral toxicity test performed prior to the beginning of the actual experiment.

3.7. **Diuretic Activity**

The method of Lahlou *et al*., (2007) was employed in the determination of diuretic activity. The rats were fasted for 18 h with free access to water. Before treatment, all animals received normal saline at an oral dose of 15 mL/1000 g body weight, to impose a uniform water and salt load (Benjumea et al., 2005). Each groups was then administered the extracts and vehicles orally by gavages. Immediately after administration, the rats were individually placed in a metabolic cage. During this period no food and water was made available to the animals. The urine was collected and measured at 1, 2, 3, 4, 5 and 24 h after dosing and stored at −20°C for electrolyte analysis.

The following parameters were determined in order to compare the effects of the extracts with vehicle and standard on urine excretion. The urinary excretion independent of the animal weight was calculated as total urinary output divided by total liquid administered (Formula −1). The ratio of urinary excretion in test group to urinary excretion in the control group was used as a measure of diuretic action of a given dose of an agent (Formula −2). A parameter (factor) known as diuretic activity was also calculated. To obtain diuretic activity, the diuretic action of the extract was compared to the diuretica action of the standard drug in the test group (Formula – 3) (Mukherjee., 2000).

$$\text{Urinary Excretion} = \frac{\text{Total urinary output}}{\text{Total liquid administered}} \times 100\% \qquad (1)$$

$$\text{Diuretic Action} = \frac{\text{Urinary excretion of treatment groups}}{\text{Urinary excretion of control group}} \qquad (2)$$

$$\textit{D}\text{iuretic Activity} = \frac{\text{Diuretic action of test group}}{\text{Diuretic action of standard group}} \qquad (3)$$

3.8. **Analytical Procedure**

Sodium, potassium and chloride levels of urine were analyzed by using Ion Selective Electrode (ISE) analyzer (AVL 9181 Electrolyte Analyzer, Roche, Germany). A calibration was performed automatically prior to analysis with different levels of standards. The ratios of electrolytes; Na^+/K^+ and $Cl^-/K^+ + Na^+$ were calculated to evaluate the saluretic activity of the different extracts. In addition, urine pH was directly determined on pooled fresh urine samples using a pH meter. Furthermore, the salt content of the extract was also determined to rule out its contribution on urinary electrolyte concentration.

3.9. **Phytochemical Screening**

Phytochemical screening tests were carried out for the aqueous and 80% methanol extracts of the plant using standard procedures to identify the presence of secondary metabolites like tannins, saponins, flavonoids, terpenoids, steroids, alkaloids and cardiac glycosides (Ajayi et al., 2011).

Test for tannins

About 1 g of each powdered sample was separately boiled with 20 mL distilled water for 5 min in a water bath and filtered while hot. Roughly 1 mL of cool filtrate was distilled with 5 mL water. A few drops of 10 % ferric chloride were added and observed for formation of precipitates or colour change. A bluish-black or brownish-green precipitate indicated the presence of tannins.

Test for saponins

About 1 g of powdered dry sample was boiled with 10 mL of distilled water in a bottle bath for 10 min. The mixture was filtered, allowed to cool and 2.5 mL of filtrate was diluted to 10 mL with distilled water and shaken vigorously for 2 min; frothing indicated the presence of saponin.

Test for terpenoids

About 5 mL of each extracts was mixed with 2 mL of chloroform and 3 mL of concentrated sulphuric acid was added to form a layer. A reddish brown precipitate coloration at the interface formed indicated the presence of terpenoids.

Test for flavonoids:

About 1 g of each extract was boiled with 10 mL of distilled water for 5 min and filtered while hot. Few drops of 20% sodium hydroxide solution were added to 1 mL of the cooled filtrate. A change to yellow colour which on addition of acid changed to colourless solution indicated the presence of flavonoids.

Test for cardiac glycosides:

Approximately 5 mL of each extracts was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 mL of concentrated sulphuric acids. A brown ring at the interface indicated the deoxy sugar characteristics of cardenolides.

Test for alkaloids:

A total of 0.5 g of each extract stirred with 5 mL of 5% queous hydrochloric acid, and heated on a steam bath when cooled, few drops of Dragendroffs reagent (potassium bismuth iodide) was added. Appearance of the reddish brown precipitate indicated the presence of alkaloids.

3.10 Statistical Analysis

Each result is presented as mean ± SEM (standard error of mean). Statistical analysis of the data were performed with one-way analysis of variance (ANOVA) followed by the Tukey post hoc comparison test. Dose dependent effects were evaluated using correlation coefficient. Significant differences were set at p- values lower than 0.05.

4. Results

4.1. Acute Toxicity Test

The rats were observed for two weeks to see if both the aqueous and 80% methanol plant extracts had an acute toxic effect. From the preliminary toxicity study, it was observed that the animals were found to be safe at a dose of 2000 mg/kg body weight. This was confirmed by lack of tremor, weight loss, paralysis, or aversive behaviors. There was no sign of diarrhea and deaths encountered with treatment of the limit dose of *Foeniculum vulgare,* indicating that the median lethal oral dose of the plant extract is greater than 2000 mg/kg.

4.2. Effect on Urine Volume

4.2.1. Aqueous Extract

The effect of oral administration of aqueous extract of *Foeniculum vulgare* on urinary output is shown in Table 1. The aqueous extract produced diuresis, which showed to be a function of dose and time ($r^2 = 0.942$; $p<0.001$). A100 did not produce any detectable difference in urine volume compared to control animals throughout the 24 h period. A200 increased diuresis starting from the 1st h of urine collection (60%, $p<0.01$) and maximum diuresis was observed at the end of 24 h (65%, $p<0.001$) when compared to control. A400 produced diuresis which was significant starting from the 1st h and maximum increase of diuresis was recorded at the end of 24 h (102%, $p<0.001$) compared to the control. HCT10 had better diuresis than A100 ($p<0.001$), a bit higher effect than A200, and comparable effect with A400 at the end of the 24 h observation period. This could be seen from the diuretic activity of A100, A200, A400 and HCT10 which were 0.56, 0.83, 1.02 and 1.00 respectively.

When different doses of the aqueous extract were compared with each other, A400 produced diuresis which was significant starting from the 1st h and continued till the end of the 24 h compared with A100. A400 produced better diuresis starting from the 1st h and continued till the end of 24 h when compared to A200. A200 increased urine volume that reached significant level starting from the 1st h ($p<0.05$) and continued up to 24 h ($p<0.001$) compared to A100.

Table 1: Effect of aqueous extracts of *Foeniculum vulgare* leaf on 24 h urine volume in rats

Group	Volume of Urine (mL)						Diuretic Action	Diuretic Activity
	1 h	2 h	3 h	4 h	5 h	24 h		
CONT	1.00 ± 0.05	1.21 ± 0.07	1.46 ± 0.04	1.80 ± 0.05	2.01 ± 0.05	4.25 ± 0.04	1.00	
HCT10	1.96 ± 0.04^{a3}	2.40 ± 0.00^{a3}	2.88 ± 0.23^{a3}	3.55 ± 0.07^{a3}	3.98 ± 0.07^{a3}	8.45 ± 0.14^{a3}	1.98	1.00
A100	1.05 ± 0.10^{b3,c3,d3}	1.53 ± 0.10^{b3,c1,d3}	1.75 ± 0.07^{b3,c1,d3}	1.98 ± 0.09^{b3,c3,d3}	2.26 ± 0.06^{b3,c3,d3}	4.68 ± 0.06^{b3,c3,d3}	1.10	0.56
A200	1.66 ± 0.05^{a3,b1,d1}	2.10 ± 0.05^{a3,d2}	2.43 ± 0.09^{a3,d1}	2.65 ± 0.13^{a3,b3,d3}	3.26 ± 0.06^{a3,b3,d3}	7.03 ± 0.18^{a3,b3,d3}	1.65	0.83
A400	2.00 ± 0.09^{a3,c1}	2.88 ± 0.25^{a3,c2}	3.08 ± 0.18^{a3,c1}	3.60 ± 0.12^{a3,c3}	4.16 ± 0.11^{a3,c3}	8.60 ± 0.06^{a3,c3}	2.02	1.02

Each value represents mean ± S.E.M (n=6) and was analyzed by ANOVA followed by Tukey post hoc multiple comparison test. [a] against control, [b] against standard, [c] against A200 mg/kg, [d] against A400 mg/kg; [1]: $p < 0.05$, [2] :$p < 0.01$, [3]: $p < 0.001$; A100: aqueous extract 100 mg/kg, A200: aqueous extract 200 mg/kg, A400: aqueous extract 400 mg/kg, HCT10: hydrochlorothiazide 10 mg/kg, CONT: animals treated with distilled water

4.2.2. **Methanol Extract**

The 80% methanol extract also produced diuresis that appeared to be a dose and time dependent manner (r^2 = 0.923; $p<0.001$). As presented in Table 2, M200 and M400 produced an increased diuresis starting from the 1st h ($p<0.001$) compared to control group. M200 ($p<0.001$) produced a lower diuretic effect as compared to HCT10, while M400 had an effect comparable to HCT10 with diuretic action of 1.93 vs 1.98 respectively.

When different doses of 80% methanol treated animals compared; M400 produced an increased diuresis which was significant starting from the 1st h, and the maximum increase had occurred at the end of 24 h ($p<0.001$) when compared with M100. M400 produced an increased diuresis starting from the 1st h ($p<0.05$) and continued till the 24 h ($p<0.001$) compared to M200. M200 produced diuresis that reached significant level starting from the 1st h ($p<0.05$) and continued up to 24 h ($p<0.001$) compared with M100.

Comparing the two extract, the aqueous extract had better diuretic activity than 80% methanol extract. A400 produced diuresis that reached significant level starting from the 1st h compared with M200 ($p<0.05$) and continued up to the end of 24 h ($p<0.001$). M400 produced diuresis that was comparable with A400. M400 found to increase urine volume that reached significant level starting from the 5th h ($p<0.001$) compared with A200.

Table 2: Effect of 80% methanol extracts of *Foeniculum vulgare* leaf on 24 h urine volume in rats

Group	Volume of Urine (mL)						Diuretic Action	Diuretic Activity
	1 h	2 h	3 h	4 h	5 h	24 h		
CONT	1.00 ± 0.05	1.21 ± 0.07	1.46 ± 0.04	1.80 ± 0.05	2.01 ± 0.05	4.25 ± 0.04	1.00	
HCT10	1.96 ± 0.04^{a3}	2.40 ± 0.00^{a3}	2.88 ± 0.23^{a3}	3.55 ± 0.07^{a3}	3.98 ± 0.07^{a3}	8.45 ± 0.14^{a3}	1.98	1.00
M100	1.03±0.05^{b3,c3,d3}	1.35±0.10 ^{b3,c1,d3}	1.66±0.10 ^{b3,c1,d2}	1.88±0.06 ^{b3,c2,d3}	2.13 ±0.04 ^{b3,c3,d3}	4.66±0.08 ^{b3,c3,d3}	1.09	0.55
M200	1.53±0.04^{a3,b3,d2}	1.93±0.08^{a2}	2.28±0.10^{a2,b1}	2.43±0.07^{a3,b3,d3}	3.01±0.07^{a3,b3,d3}	6.95±0.09^{a3,b3,d3}	1.63	0.82
M400	1.88±0.04^{a3,c2}	2.33±0.04^{a3}	2.53±0.13^{a3}	2.86±0.08^{a3,c3}	3.86±0.09^{a3,c3}	8.20±0.14^{a3,c3}	1.93	0.97

Each value represents mean ± S.E.M (n=6) and was analyzed by ANOVA followed by Tukey post hoc multiple comparison test. [a] against control, [b] against standard, [c] against M200 mg/kg, [d] against M400 mg/kg; [1]: $p < 0.05$, [2] :$p < 0.01$, [3]: $p < 0.001$; M100: 80% methanol extract 100 mg/kg, A200: 80% methanol extract 200 mg/kg, M400: 80% methanol extract 400 mg/kg, HCT10: hydrochlorothiazide 10 mg/kg, CONT: animals treated with distilled water

4.3. **Effect on Electrolyte Content of the Urine**

4.3.1. **Aqueous Extract**

The cumulative urine volume collected in the 24 h were analyzed for the electrolyte contents (Na^+, K^+, and Cl^-) and the results presented in Table 3. A200 increased sodium excretion by 43% which was significant compared to the control groups. By contrast, A400 significantly increased sodium excretion by 60% compared to the controls. HCT10 increased sodium excretion by 67% which was significant when compared with control group.

Urinary potassium excretion profiles was measured for all the entire treatment groups and the controls. A200 caused an increased potassium excretion (30%) and A400 cause the maximum loss of potassium (35%) which was found to be significant compared to the control group.

The aqueous extract doses increased urinary chloride excretion which was significant (50% and 68% respectively for A200 and A400) compared to control. As indicated in Table 3, the saluretic indices of Na^+ and Cl^- of the aqueous extracts at the highest dose and HCT10 were comparable (1.60, 1.67 vs 1.68, 1.69). In addition, the Na^+/K^+ ratio of A400 was higher than the ratio for HCT10 (1.78 vs 1.73). From the calculated $Cl^-/Na^+ + K^+$ ratios, A100 had the lowest value (0.59).

Comparing the different doses of the aqueous extract, A400 and A200 had produced a significant natriuresis as compared to A100 with ($p<0.001$) for both doses. A400 had the highest kaliuresis effect as compared to A100, but no significant difference when compared with A200. The excretion profile of chloride, however, showed a significant difference in between the doses.

Table 3**:** Effect of aqueous extracts of *Foeniculum vulgare* leaf on urinary electrolyte excretion in rats

Group	Urinary Electrolyte Concentration (mmol/L)			Saluretic Index			Na^+/K^+	$Cl^-/Na^+ + K^+$
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-		
CONT	70.50 ± 1.87	46.83 ± 1.87	65.67 ± 1.67				1.50	0.50
HCT10	118.17 ± 1.13^{a3}	68.17 ± 2.32^{a3}	111.17 ± 8.08^{a3}	1.67	1.97	1.69	1.73	0.60
A100	$87.83 \pm 1.94^{b3,c3,d3}$	$52.33 \pm 1.63^{b3,c3,d3}$	$82.33 \pm 3.67^{b3,c3,d3}$	1.25	1.52	1.25	1.67	0.59
A200	$101.16 \pm 1.67^{a3,b3,d3}$	$60.83 \pm 2.13^{a3,b3}$	$98.50 \pm 2.04^{a3,b3,d3}$	1.43	1.76	1.37	1.67	0.60
A400	$112.83 \pm 4.88^{a3,c3}$	$63.33 \pm 3.61^{a3,b1}$	$110.33 \pm 2.94^{a3,c3}$	1.60	1.83	1.68	1.78	0.62

Each value represents mean ± S.E.M (n=6) and was analyzed by ANOVA followed by Tukey post hoc multiple comparison test. [a] against control, [b] against standard, [c] against A200 mg/kg, [d] against A400 mg/kg; [1]: $p < 0.05$, [2]: $p < 0.01$, [3]: $p < 0.001$; A100: aqueous extract 100 mg/kg, A200: aqueous extract 200 mg/kg, A400: aqueous extract 400 mg/kg, HCT10: hydrochlorothiazide 10 mg/kg, CONT: animals treated with distilled water

4.3.2. **Methanol Extract**

The urinary sodium excretion showed an increasing pattern as it was 14%, 30% and 54% for the respective doses of M100, M200 and M400 when compared with control. K^+ excretion was also increased from M100 to M400 compared to control. The Cl^- excretion profile had also showed an increasing manner of 18%, 37% and 63% for M100, M200, and M400 as dipected in Table 4.

The sodium excretion of M100 and M200 was lesser as compared to HCT10, but M400 have had comparable effects. The K^+ excretions for all doses were lower than HCT10. In case of chloride excretion, there was significant difference between the first two doses and HCT10. M400 and HCT10 had comparable effect on chloride excretion. The saluretic indices had also calculated and nearly closer results were obtained for Na^+ and Cl^- between M400 and HCT10 (1.54, 1.67 vs 1.63, 1.69). From the calculated $Cl^-/Na^+ + K^+$ ratio, M100 provided the lowest value of 0.61.

When the different doses of 80% methanol extract were compared, both M200 and M400 had resulted in significant Na^+ excretion compared to M100 ($p<0.001$) for both doses. M400 had the highest kaliuresis compared to M100 and M200 ($p<0.001$). M400 also caused chloride excretion that reached significance level when compared with M100 and M200 ($p<0.001$).

Comparing the two extract, A400 increased urinary electrolytes that reached significant level ($p<0.001$) compared to M100 and M200 for all the three electrolytes. M400 increased urinary electrolytes ($p<0.001$) compared to A100. There was no significant difference between M400 and A200 on potassium excretion. But M400 was found to cause loss of sodium and chloride ion that reached significant level ($p<0.01$, $p<0.05$ respectively) compared to A200. A200 increased urinary electrolytes that reached significant level ($p<0.001$) compared to M200.

4.3.3. **Electrolyte Content of the Extract**

Water soluble salts could be present in the extract and consequently interfere with the urinary excretion of electrolytes. The content of Na^+, K^+ and Cl^- both in aqueous and 80% methanol extracts was determined to exclude this possibility of interference. The result revealed that there were no detectable levels of the three electrolytes (Na^+, K^+, Cl^-) at all doses in the extracts as tested by ISE analyzer.

Table 4: Effect of 80% methanol extracts of *Foeniculum vulgare* leaf on urinary electrolyte excretion in rats

Group	Urinary Electrolyte Concentration (mmol/L)			Saluretic Index			Na^+/K^+	Cl^-/Na^++K^+
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-		
CONT	70.50 ± 1.87	46.83 ± 1.87	65.67 ± 1.67				1.50	0.50
HCT10	118.17 ± 1.13^{a3}	68.17 ± 2.32^{a3}	111.17 ± 8.08^{a3}	1.67	1.97	1.69	1.73	0.60
M100	80.67 ± 1.97^{b3,c3,d3}	46.67 ± 2.58^{b3,c3,d3}	78.00 ± 1.90^{b3,c3,d3}	1.14	1.35	1.18	1.73	0.61
M200	91.67 ± 3.26^{a3,b3,d3}	53.83 ± 1.94^{a3,b3,d3}	90.00 ± 2.45^{a3,b3,d3}	1.30	1.56	1.37	1.70	0.62
M400	109.17 ± 6.79^{a3,c3}	62.33 ± 2.50$^{a3, b2,c3}$	107.33 ± 4.90^{a3,c3}	1.54	1.80	1.63	1.75	0.62

Each value represents mean ± S.E.M (n=6) and was analyzed by ANOVA followed by Tukey post hoc multiple comparison test. [a] against control, [b] against standard, [c] against M200 mg/kg, [d] against M400 mg/kg; [1]: $p < 0.05$, [2] :$p < 0.01$, [3]: $p < 0.000$; M100: 80% methanol extract 100 mg/kg, A200: 80% methanol extract 200 mg/kg, M400: 80% methanol extract 400 mg/kg, HCT10: hydrochlorothiazide 10 mg/kg, CONT: animals treated with distilled water

4.4. **Urinary pH**

The urinary pH was measured and the different treatment groups of both aqueous and 80% methanol had resulted in different urine pH.

4.4.1. **Aqueous Extract**

Urinary pH measurement revealed that the different treatment groups of aqueous extracts had produced relatively alkaline urine as shown in Figure 4. The pH of rats' urine treated with the aqueous extract had shown an increasing order from A100 (8.20) to A400 (8.45). The control had produced the lowest pH and the standard gave rise to alkaline urine like the aqueous extract. But there was not any significant difference between the extracts and the controls.

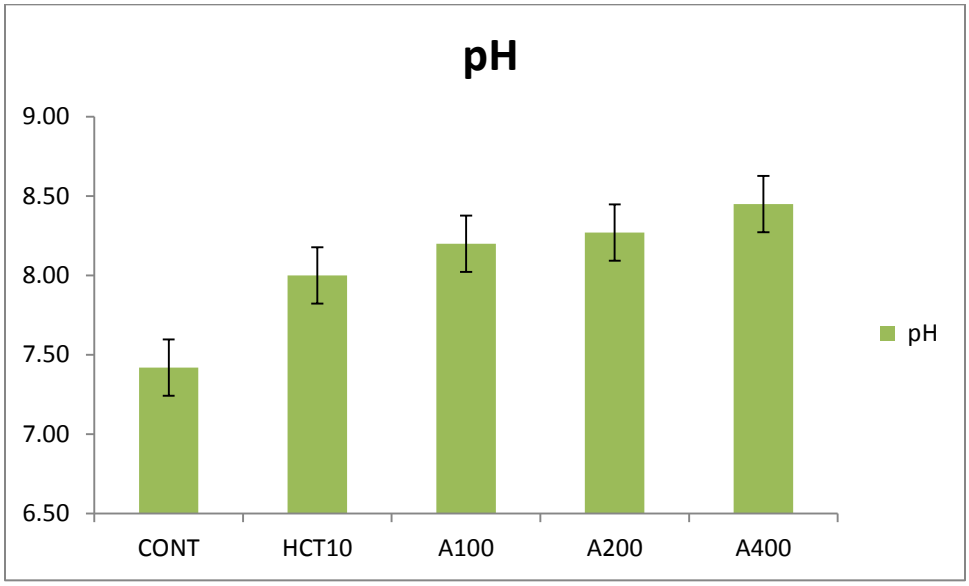

Figure 4: Urinary pH of rats teated with the aqueous leaf extract of *Foeniculum vulgare*

4.4.2. **Methanol Extract**

The rats treated with the 80% methanol extract produced slightly less alkaline urine compared to the control which was in the range of 7.00 (M100) to 7.50 (M400). M100 produced the lowest pH and M400 produced slightly the alkaline urine, but the difference in between groups found to be insignificant.

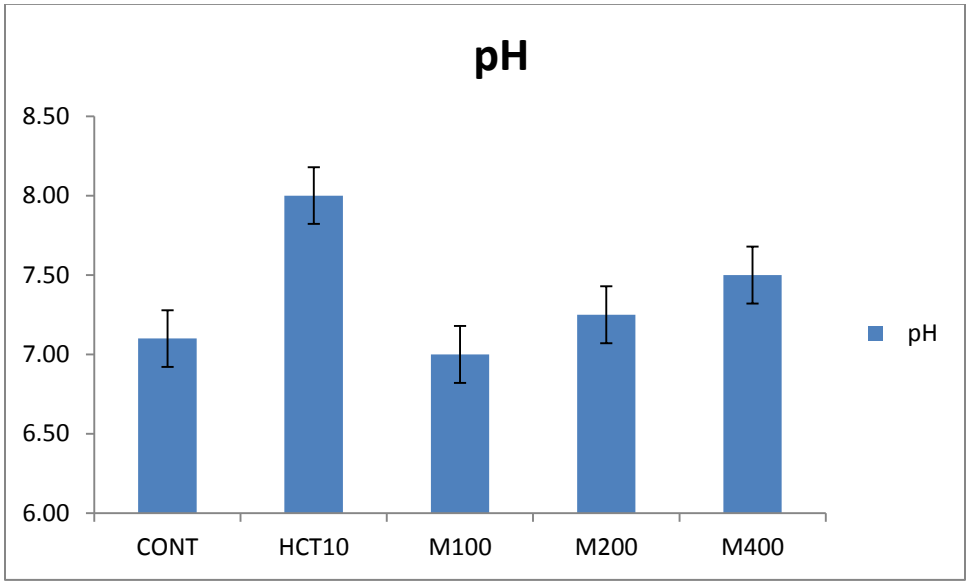

Figure 5: Urinary pH of rats treated with 80% methanol leaf extract of *Foeniculum vulgare*

4.5. **Phytochemical Screening**

Both extracts of *Foeniculum vulgare* were plant investigated for the composition of secondary metabolites. Both the aqueous and 80% methanol extracts of *Foeniculum vulgare* were shown to possess tannins, saponins, flavonoids, terpenoids, steroids, alkaloids and cardiac glycosides.

Table 5: Phytochemical screening of aqueous and 80% methanol extract of *Foeniculum vulgare*

S.NO	Constituents	Aqueous extract	80% Methanol extract
1.	Alkaloids	+	+
2.	Cardiac glycosides	+	+
3.	Flavonoids	+	+
4.	Saponins	+	+
5.	Steroids	+	+
6.	Tannins	+	+
7.	Terpenoids	+	+

+ = present

5. **Discussion**

Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Since diuretics are employed clinically in the treatment of edema (Jackson., 2006), it would be highly imperative to demonstrate effectiveness of the plant extracts in the presence of electrolytes and water. In view of urine output, both the aqueous and 80% methanol extract showed an increase in diuresis that appeared to vary with dose and time. Compared to the 80% methanol extract, the aqueous extract produced a better diuretic effect. This difference in their diuretic activity could be seen in different doses used in the experiment. The lower doses of both 80% methanol and aqueous extracts did not produce visible effect throughout the experiment, but the medium dose of both extract was able to produce significant effect from the beginning. This could probably suggest that the lower doses of both extract might represent minimum doses that cannot elicit diuresis. Increasing the dose did affect the diuretic effect produced by the plant extracts. For instance, the diuretic effects of A400 was higher than the diuretic effects of A200 (4.16±0.11 vs 3.26±0.06) after 5 h (Table 1). Moreover, the diuretic activity (0.83) of A200 was lower than that of A400 (1.02). In contrast, M400 produced a diuretic effect of (3.86±0.09) which was yet lower than the diuretic effect of A400 after 5 h. So, it is possible to suggest that the ingredient(s) of the plant responsible for diuretic effect probably polar and better extracted by water than 80% methanol.

The diuretic activity of the extracts of *Foeniculum vulgare* at their higher corresponding doses was a moderate type for the aqueous and mild for the 80% methanol extract, as their values were 1.02 and 0.97 for A400 and M400 respectively. The diuretic activity is considered to be good if

the diuretic activity value is greater than 1.50, moderate if the value is between 1.00 and 1.50, mild if the value lies between 0.72 and 1.00, and nil if the value is < 0.72 (Vogel., 2007).

The effect of the extract on water excretion was accompanied by urinary electrolyte excretion effect, which supports the idea that the diuretic effect of *Foeniculum vulgare* was of the saluretic type in contrast to aquaretic types, which is a typical features of most the phytodiuretic agents (Mekonnen et al., 2010; Praveen et al., 2013). The middle and higher doses of both extracts had a remarkable natriuretic effect compared to the control and thus could have a favorable effect in different edematous conditions. The ratios of Na^+/K^+ was calculated as indicator of natriuretic activity and found to be 1.78, 1.75 and 1.73 for A400, M400 and HCT10 respectively.This indicates that the extracts increased both sodium and potassium excretions compared to control groups. In contrast to previous study of aqueous and methanol extracts of some plants like *Ajuga remota* (Hailu et al., 2014), which showed an interesting K^+ saving effect at the higher doses, this plant is devoid of such effects at all doses for both the aqueous and methanol extracts. However, this finding is in line with the aqueous and 80% methanol extracts of Rumex abyssinicus J (Mekonnen et al., 2010) and aqueous ethanol extract of leaves of *Costus speciosusin* (Prabhu et al., 2014), which demonstrated to be devoid of K^+ sparing effect.

Moreover, onset of the diuretic action of the middle and higher doses of the aqueous extract were sufficiently rapid 1-2 h) compared to the methanol extract and had a fairly long duration of action as it produced significant effect from the 1^{st} h ($p<0.001$) to the 24 h ($p<0.001$) experiment period, indicating fast absorption from the gastrointestinal tract and slow clearance of the extract from the body. This is an interesting diuretic profile, as it would decrease the frequency of administration.

Thiazide and thiazide like diuretics including hydrochlorothiazide increase urinary flow rate and urinary electrolyte excretion specially sodium, potassium and chloride, by interfering with Na^+-Cl^- symporter (co-transporter) activity in the distal tubule and also to some extent by inhibiting carbonic anhydrase enzyme in the proximal renal tubule (Hullatti et al., 2014). Because the aqueous and 80% methanol extracts of *Foeniculum vulgare* produced both water and electrolyte excretion, it is possible that the extracts exerted diuretic effect by inhibiting tubular reabsorption of water and electrolytes as such action has been suggested for some other plants (Prabhu et al., 2014). The possibility of direct action of potassium content of the plant extract on diuretic effect could be excluded, since the K^+ content of the extract was very low and undetectable for all doses of both for aqueous and methanol extracts.

The onset of diuretic action was quick, the effect was long lasting and the urine was markedly hyperkalemic and alkaline as indicated by K^+ level in the urine and pH of urine, respectively. These observations suggested that both extracts of *Foeniculum vulgare* are not acting as potassium sparing diuretics: potassium-sparing diuretics are usually have slow onset of action and increase the urinary Na^+/K^+ ratio (Jayakody et al., 2011). The larger doses of aqueous and 80% methanol extracts of *Foeniculum vulgare* used in this study produced similar Na^+ and Cl^- excretion profile to that of hydrochlorothiazide. This could possibly suggest that the mechanism by which the extract produces diuresis is similar to that of thiazide diuretics.

The $Cl^-/ Na^+ + K^+$ ratio was calculated and shows the extent of carbonic anhydrase inhibitory effect. Carbonic anhydrase inhibition can be excluded at ratios between 0.8 and 1.0 and with decreasing ratios; slight to strong inhibition can be assumed (Vogel., 2007). The $Cl^-/ Na^+ + K^+$ ratio was calculated for both extracts and showed slight carbonic anhydrase inhibitory effect with

values ranging from 0.59 – 0.62. Thus, it appears likely that carbonic anhydrase inhibition effect of the plant extract might have contributed to the highest K^+ loss compared to the other plants (Mekonnen et al., 2010). From this observation, it is reasonable to assume one of the possible mechanism of action of these extracts could be carbonic anhydrase inhibition.

In determination of urinary pH, the aqueous extracts showed a relative increase in pH values as compared to controls, reinforcing the notion that carbonic anhydrase inhibition as one of the possible mechanism of action of the plant. In addition, sodium and chloride excretion profile of both extracts was comparable to the standard hydrochlorothiazide which increased Na^+/Cl^- ratio which is called thiazide secretory index (Jayakody et al., 2011). This observation suggests a thiazide like mode of action as a possible mechanism of action of the plant. Thiazide type of diuretics elevate thiazide secretory index, simultaneously by increasing urinary levels of sodium and potassium by inhibiting Na^+/Cl^- co-transporter in the distal convoluted tubule of the nephron.

The exact nature of the active ingredient(s) responsible for the diuretic effects of the 80% methanol and aqueous extracts of this plant is/are not known so far. Nonetheless, preliminary phytochemical screening carried out with both extracts revealed the presence of bioactive molecules including alkaloids, flavonoids, saponnins, tannins and steroids. Previous studies (Sayana et al., 2014; Asif et al., 2014) have demonstrated that there are several compounds which could be responsible for the plants diuretic effects like flavonoids, saponins or organic acids. The effect may be produced by stimulating regional blood flow or inhibition of tubular reabsorption of water and ions (Reddy et al., 2011), with the result in all cases being diuresis.

To sum up, this study supports the traditional use of *Foeniculum vulgare* for its diuretic effect. Based on the pattern of urine excretion, sodium and potassium, it appears that the plant might possibly have more than one mechanism of action which contributes to the observed saluretic effect. Multiple mode of action had been reported with some herbal medicines (Martin-Herreraa et al., 2008). Hence, adding up to the anticipated carbonic anhydrase inhibitory effect, there must be another mode of action that contribute to the highest diuretic effect of the plant.

6. **Conclusions**

This study provides support for the traditional use of *Foeniculum vulgare* as a diuretic agent through enhancement of salt and water excretion. Although the active components (s) which is responsible for the observed effect remain(s) to be seen, polar constituents singly or in synergy act by multiple mechanisms to produce the observed effect. Looking at the data, the larger doses, of the aqueous extract produced a very interesting diuresis profile, which was comparable to hydrochlorothiazide. From the electrolytes analysis and urinary pH it is plausible to assume that the plant could have multiple mode of action. In the present study, no lethality was observed at least for the dose and duration used, which could show that safety profile of the extract as an added advantage. Finally, it is evident from the experiment that the aqueous and 80% methanol extracts of *Foeniculum vulgare* leaves have showed an increase in urine and electrolyte excretion which supports its traditional use as a diuretic agent.

7. **Recommendation**

- ➢ Investigation of specific component(s) responsible for the diuresis should be analyzed from the different fractions of the crude extracts
- ➢ It remains necessary to study chronic toxicity and eventual adverse effect(s) of this plant such as alteration of neural, metabolic and hormonal parameters, which are undetermined in this study
- ➢ The precise site(s) of action, the molecular and cellular mechanism(s) of *Foeniculum vulgare* action remain to be elucidated in further studies.

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