Addis Ababa University

College of Health Sciences

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Evaluation of the Diuretic Activity of the Aqueous and 80% Methanol Leaves Extracts of *Ficus Sur* Forssk (Moraceae) in Rats

By

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This is to certify that the thesis prepared by Mesfin Ayele, titled with: Evaluation of the Diuretic Activity of the Aqueous and 80% Methanol Leaves Extracts of *Ficus sur* Forssk (Moraceae) in Rats and submitted in partial fulfillment of the requirements for the degree of Master of Science 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 the Aqueous and 80% Methanol Leaves Extracts of *Ficus Sur* Forssk (Moraceae) in Rats

Mesfin Ayele

Addis Ababa University, 2020

Ficus sur Forssk (Moraceae), commonly known as cape fig, is traditionally used in treatment of many diseases and ailments. Although investigations on different pharmacological activities of the plant have been conducted, its folklore use for diuresis has not yet been studied. The current study, therefore, Ficused on the diuretic activity of aqueous and 80% methanol leaves extracts of *Ficus sur* in rats. Rats were randomly assigned into eight groups each consisting of six rats for diuretic test. The negative control group was treated with distilled water (2 mL/100gm); whilst positive control group was treated with furosemide (10 mg/kg). The doses (100mg/kg, 200mg/kg, and 400mg/kg) of aqueous and 80% methanol extracts were administered to rats in group III-VIII, and urine volume was recorded every hour until the end of the fifth hour, and cumulative urine volume of each rat was taken. Then, diuretic activity, diuretic index, saliuretic index, natriuretic index and carbonic anhydrase inhibition index were calculated to make comparison among the groups. As an electrolyte excretion of all doses of the extracts showed significant natriuresis ($p<0.001$) and chloriuresis ($p<0.01$), the middle (200mg/kg) and the highest (400mg/kg) doses of both the aqueous and 80% methanol extracts significantly increased diuresis at the fifth hour ($p<0.001$) compared to the negative control, though it was less than that of the positive control. In addition, aqueous extract displayed more significant diuretic effect than 80% methanol extract. The aqueous and 80% methanol extracts produced alkaline urine. The crude leaves extracts of *Ficus sur* increased urinary excretion and concentration of urinary electrolytes in a dose-dependent manner. These findings uphold the traditional use of *Ficus sur* as diuretic agent as claimed by traditional healers.

Key Words: Ficus sur, Diuretic activities, Urinary electrolyte, Furosemide, Rat, Aqueous extract, 80% Methanol extract.

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Lists of Abbreviations and Acronyms

ADH	Anti-Diuretic Hormone
ARs	Adenosine Receptors
AVP	Arginine Vasopressin
CD	Collecting Duct
CHF	Congestive Heart Failure
DCT	Distal Convoluted Tubule
DW	Distilled Water
ENaC	Epithelial Na^+ Channel
FURO10	Furosemide 10mg/kg
GPCRs	G-protein Coupled Receptors
GFR	Glomerular Filtration Rate
IMCD	Inner Medullary Collecting Duct
JGA	Juxtaglomerular Apparatus
MD	Macula Densa
PCT	Proximal Convoluted Tubule
PLC	Phosphatidylinositol
UT	Urea Transporters
TGF	TubuloGlomerular Feedback
VRs	Vasopressin Receptors

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1. Introduction

1.1. History of diuretics

The term diuresis is derived from the Greek word ‘diuretikos’, which means ‘prompting urine’. Diuretics are drugs that increase the rate of formation of urine which cause a net loss of sodium and water (Hullatti *et al.*, 2011). ‘Natriuretic’ refers to chemicals that increase renal sodium excretion while the term aquaretics refer to agents that increase renal excretion of free water (Lang & Hropot, 1995).

Many compounds had been investigated for their diuretic activities. Mercurial diuretics were a major advance starting from the time of ancient Rome to manage edema, purgatives, blood-letting, and paracentesis. For instance, mercuric chloride was known to have diuretic properties since 1553, Paracelsus recorded it as a cure for dropsy. Despite tremendous efforts, only few effective treatments existed until 1918, Alfred Vogl serendipitously discovered an organic mercurial agent called merbaphen that dramatically increased urine output. But, they had serious side effects including stomatitis, colitis, proteinuria, and hematuria (Wile, 2012).The discovery of sulfanilamide led to the development of acetazolamide that caused metabolic acidosis and natriuresis. Subsequent efforts to modify sulfonamide-based drugs was led to the development of chlorothiazide in 1957 and later to the development of furosemide (Lang & Hropot, 1995). Ethacrynic acid was synthesized in a thoughtful attempt to mimic the sulfhydryl reactivity of mercurial diuretics (Abraham *et al.*, 1989). Later on, the search for potassium sparing diuretics resulted in the introduction of spironolactone, amiloride and triameterene (Zawada, 1986).

1.2. Renal anatomy and physiology

Most people have two kidneys. However, it is possible to live an active and healthy life with only one functioning kidney. In rare instances people can be born with three kidneys, and likewise remain healthy (Marieb & Hoehn, 2007). The kidneys are positioned on either side of the spine just below the ribs. It contains gross landmarks: the cortex, medulla, renal papilla, and renal pelvis (Pallabi, 2018). The kidneys are the main organs of homeostasis that maintain the acid-base and water salt balance of the blood which is regulated mainly through renin angiotensin aldosterone system (RAAS) and anti-diuretic hormone (ADH) (Soleimani & Rastegar, 2016). Each kidney

receives its blood from a single renal artery and then the urine formed is drained via a single ureter into the bladder (Knepper & Stephenson, 1987).

Kidney consists of two types of nephrons namely the Cortical and Juxtamedullary nephrons. Cortical nephrons are short nephron loops with peritubular capillaries while juxtamedullary nephrons are very long nephron loops with vasa recta (Jamison, 1987). Nephron is the urine-forming unit of the kidney. Basically, nephron is composed of renal corpuscle and renal tubules. Renal corpuscle is formed by a tuft of capillaries called glomerulus and epithelium of the Bowman's capsule (Ojeda *et al.*, 2003). Renal tubule consists of proximal convoluted tubule (PCT), loop of Henle, distal convoluted tubule (DCT) and Collecting duct (CD). Filtration occurs in renal corpuscle so that water, salts, small molecules and wastes are filtered out of the blood. During urine formation the initial filtrate is modified by a variety of secretory and reabsorptive processes as it passes through PCT, Loop of Henle, DCT and CD (Brenner *et al.*, 1968). Besides, different substances are reabsorbed back into blood from different parts of the tubule; in PCT ~80% of materials can be reabsorbed while in DCT and CD additional water is reabsorbed under the control of ADH (McDonald *et al.*, 1976).

Further, the specialized epithelial cell of thick ascending limb of the loop of Henle has masses of cells called macula densa (MD) which are in contact with cells of the afferent arterioles called juxtaglomerular cells. Together with extraglomerular mesangial cells, they form juxtaglomerular apparatus (JGA) (Tobian, 1962). Extraglomerular mesangial cells are interconnected by gap junctions and pass signals between MD and juxtaglomerular cells. The MD cells are chemoreceptors that respond to changes in the NaCl content of the filtrate to elicit compensatory adjustments. A high NaCl at this site causes contraction of the afferent arteriole and a reduction in glomerular filtration rate (GFR). On the other hand, juxtaglomerular cells are renin containing secretory granules that sense the blood pressure in the afferent arterioles (Schnermann, 2003). This renal hemodynamics regulation of JGA is referred to as tubuloglomerular feedback (TGF) (Blantz & Pelayo, 1984; Ploth *et al.*, 1979).

1.3. Mechanism and site of actions of conventional diuretics

Unlike aldosterone receptor blockers, all diuretic agents must reach the lumen of the renal tubule and produce diuresis primarily by inhibiting Na^+ reabsorption at different segments of renal tubules

which in turn decreases the reabsorption of Cl^- and water (Figure 1). This reabsorption of NaCl in renal tubules is driven by the Na^+-K^+-ATPase pump, of the basolateral membrane of tubular epithelial cells, to move Na^+ from the cell into the interstitium then to blood and K^+ from the interstitium to the cell. Therefore, these drugs are primarily saluretics and secondarily diuretics (Jacobson & Kokko, 1976; Materson, 1983). The combination of two diuretics may be given for the purpose of blocking multiple nephron sites that results in synergized diuretic effect (Jentzer *et al.*, 2010). Most diuretics are actively secreted in to PCT through the organic acid pathway.

Most commonly administered diuretics are categorized under five main classes (Setaro & Moser, 2007). These are carbonic anhydrase inhibitors, osmotic diuretics, loop diuretics, thiazide diuretics and potassium sparing diuretics.

Carbonic anhydrase inhibitors cause Na^+ dependent bicarbonate loss at PCT resulting in greater $HCO3^-$, Na^+, and water loss in the urine. At this site Na^+ reabsorption is accompanied by a luminal Na^+/H^+ antiporter (Cogan & Alpern, 1984)**.** They are the weakest of all diuretics and acetazolamide is a prototype drug (Bagga & Sinha, 2007).

Mannitol can stay within the vascular space so that it is filtered freely via the glomerulus and reabsorbed very poorly exert an osmotic diuresis (Mathisen *et al.*, 1981).

Loop diuretics such as furosemide, and torsemide are about 90% bound to plasma proteins (Kirchner *et al.*, 1991; Sarafidis *et al.*, 2010a). They inhibit Na^+-K^+-$2Cl^-$ cotransporter 2 (NKCC2) in the apical membrane of the thick ascending limb of the loop of Henle. This transporter reabsorbs about 25% of the sodium load. Apart from this, K^+ is returned in to the lumen via K^+-channels (Smith, 2014b; Wargo & Banta, 2009). This causes the Mg^+ and Ca^+ to be repelled from luminal side to interstitial side at the thick ascending limb (Blaine *et al.*, 2015; Smith, 2014b).

Thiazide diuretics inhibit Na^+-Cl^- cotransporter (NCCT) in the apical membrane of the early segment of DCT. This transporter normally reabsorbs only about 5%-10% of filtered sodium (Tamargo *et al.*, 2014). The thiazide diuretics create low Na^+ inside the cell that can stimulate basolateral Na^+/Ca^{2+} exchanger resulting in low intracellular Ca^+. This in turn stimulate the driving force for the reabsorption of Ca^+ from the lumen into the cell via apical Ca^{2+} selective channel, transient receptor potential cation channel subfamily vanilloid member 5 (TRPV5) (Magyar *et al.*,

2002). Thiazides include the prototype hydrochlorothiazide and quinethazone, metolazone, chlorthalidone and indapamide (Nijenhuis *et al.*, 2003).

The fifth class of diuretics is potassium-sparing diuretics. Sodium reabsorption at CD is mediated by epithelial Na^+ channel (ENaC) and regulated by aldosterone (Pearce *et al.*, 2015; Roy *et al.*, 2015). Potassium-sparing diuretics include spironolactone and eplerenone that inhibit aldosterone receptor and triamterene and amiloride that directly inhibit ENaCs recruited by aldosterone. As these diuretics have relatively weak effects on overall sodium balance, they are often used in conjunction with thiazide or loop diuretics to prevent hypokalemia (Catena *et al.*, 2012). It is noteworthy that carbonic anhydrase inhibitors, loop and thiazide diuretics increase sodium delivery to the late distal tubule that lead to stimulation of aldosterone-sensitive Na^+/K^+ and Na^+/H^+ exchanger leading to hypokalemia and metabolic alkalosis respectively (Soleimani & Rastegar, 2016).

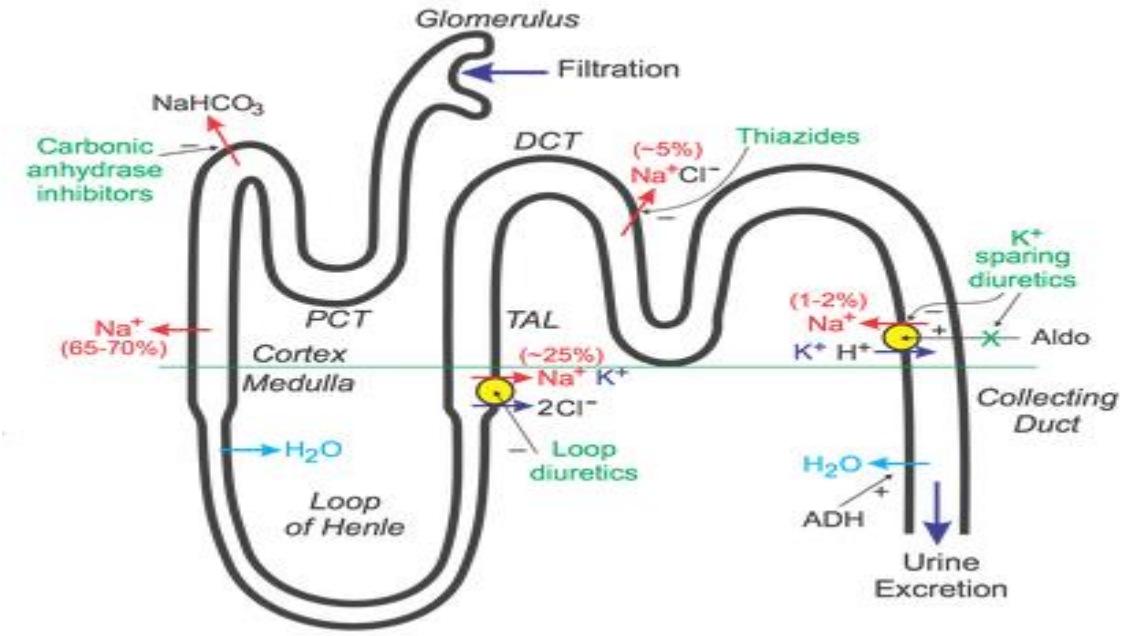

Figure 1:Major site of diuretic action in renal tubule (Smith, 2014a))

1.4. Therapeutic importance of diuretics

Diuretics are beneficial in many disease conditions such as congestive heart failure (CHF), nephritic syndrome, hypertension, liver cirrhosis, poisoning, and certain kidney diseases (Gupta & Neyses, 2005). Some diuretics, like acetazolamide , is useful for treatment of glaucoma, metabolic alkalosis and to make the urine alkaline (Morgan & Polak, 1969). Mannitol is used widely for management of cerebral oedema and acute kidney injury (Better *et al.*, 1997). Loop diuretics are usually the first choice for oedematous disorders like cardiac failure, nephrotic syndrome, hepatic cirrhosis. They also maintain or increase GFR even in the case of volume depletion (Sarafidis *et al.*, 2010b). Thiazides commonly used for hypertension, hypercalciuria, diabetes insipidus, mild oedema. The antihypertensive action of thiazides is due to chronic decrease in peripheral vascular resistance and occur at lower dose than that required to produce diuresis (Roush & Sica, 2016). Indapamide and chlorthalidone were specifically designed with this in mind and has a larger therapeutic window for hypertension than most other diuretics (Magrini, 2008). Potassium-retaining diuretics mostly used in essential hypertension in combination with other diuretics to avoid potassium deficiency (Sarafidis *et al.*, 2010a).

1.5. Adverse effects of conventional diuretics

An adverse drug reactions (ADRs) occur almost daily in health care institutions and often causing considerable morbidity and mortality (Prichard *et al.*, 1992). The common side effects associated with diuretics are electrolyte and water disturbances; these are, hypokalemia /hyperkalemia, hyponatremia, hypovolemia and acid-base imbalance. Loop and thiazide diuretics can cause hypokalemic metabolic alkalosis, hypotension, and allergic reactions (Sica, 2004). In addition to this, loop diuretics exhibit short half-life and bioavailability issue requiring repeated and large dosing. And, if left uncorrected rebound Na^+ retention could followed (Sarafidis *et al.*, 2010a). Carbonic anhydrase inhibitors can cause hyperchloremic metabolic acidosis due to increased bicarbonate excretion and inhibition of titratable acid and ammonia secretion (net acidic excretion) (Soleimani & Rastegar, 2016). Most diuretics increase elimination of potassium, resulting in the risk of serious heart rhythm disturbances. Potassium-sparing diuretics also cause hyperkalemia specially in people who already have a high potassium level or in those who have kidney disease (Viera & Wouk, 2015).

1.6. Novel diuretics

Long-term use of conventional diuretics could have several adverse effects including electrolyte disorders, hyperuricemia, hyperlipidemia, glucose tolerance and allergies. Of these for example, electrolyte abnormalities can induce cardiac arrhythmias and sudden death. Therefore, it could be desirable to discover novel diuretic targets and develop diuretics that do not cause electrolyte disturbances (Yang *et al.*, 2012). Adenosine receptor antagonists, vasopressin receptor antagonists and urea transporter inhibitors are the novel classes of diuretics (Dohi & Ito, 2014).

1.6.1 Adenosine receptor antagonists

Adenosine receptors (ARs) or purinergic1 receptors (P_1Rs) are a class of G-protein coupled receptors (GPCRs) with adenosine as endogenous ligand. There are four known ARs in humans: A_1R, A_2AR, A_2BR and A_3R; each encoded by different genes. The A_1R has been found to be ubiquitous throughout the entire body particularly in smooth muscle. This receptor has an inhibitory function on most of the tissues on which it rests (Fredholm *et al.*, 2011; Rieg *et al.*, 2005). The TGF mechanism detects flow dependent alterations of luminal NaCl causing a basolateral release of adenosine from the MD cells. Signals are then, sent by the MD to constrict afferent arterioles. During signaling Adenosine triphosphate (ATP), the MD cell Ca^+ concentration, and nitric oxide (NO) play crucial role (Ren *et al.*, 2000). This cascade of events decreases GFR that ultimately brings to an appropriate level. On the other hand, it inhibits release of renin. (Brown *et al.*, 2001; Liu, 2002). A_1R antagonists induce eukaliuretic natriuresis and diuresis by blocking A1R-mediated NaCl reabsorption in the distal tubule (Givertz *et al.*, 2007). In addition, A_1R blockade can result in vasodilatory effect and could protect renal ischaemia (Hocher *et al.*, 2011; Vallon *et al.*, 2008). An AR antagonist include caffeine, theophylline, and theobromine. Therefore, blockade of ARs may provide such novel therapy.

1.6.2 Vasopressin receptor antagonists

Vasopressin, also known as antidiuretic hormone, arginine vasopressin (AVP), is a hormone synthesized in the hypothalamus as a peptide prohormone. It travels down the axon and released from vesicles into the circulation in response to extracellular fluid hypertonicity. The actions of v asopressin are mediated by tissue-specific GPCRs called vasopressin receptors (VRs) that are

classified into V_1R (V_{1A}), V_2R, and V_3R (V_{1B}) subtypes (Maybauer *et al.*, 2008). These three subtypes differ in localization, and signal transduction mechanisms as shown in (Table 1).

Table 1:Localization, and signal transduction mechanisms of receptor subtypes (Raychowdhury *et al.*, 2009; Thibonnier *et al.*, 1999)).

Gene	Symbols	Signaling pathways	Locations
AVPR1A	V1	G protein coupled phosphatidylinositol (PLC)/Ca+	Vascular smooth muscle Dens in vasa recta, Platelet Hepatocytes, Myometrium
AVPR1B	V3	G protein coupled PLC/Ca+	Anterior pituitary gland
AVPR2	V2	Adenylyl cyclase/ cyclic adenine Monophosphate	Basolateral membrane of collecting duct, Vascular endothelium Vascular smooth muscle cell

The well-known antidiuretic effect of vasopressin occurs via activation of V_2R and through vasopressin-regulated water channel, Aquaporin-2 (AQP2), on the apical membrane of CD (Judith Radin *et al.*, 2012). Action of vasopressin on V_1R reduce blood flow in to inner medulla without affecting blood flow to outer medulla (Kinter *et al.*, 1993). The VR antagonist commonly used to treat hyponatremia caused by syndrome of inappropriate antidiuretic hormone secretion (SIADH), CHF and cirrhosis (Maybauer *et al.*, 2008). They include "vaptan drugs" such as conivaptan (non-selective), tolvaptan Food and Drug Administration (FDA) approved, mozavaptan, lixivaptan, satavaptan (Lehrich *et al.*, 2013).

1.6.3 Urea transporter inhibitors

In mammalian cells, urea is generated by the liver as chief end-product of nitrogen catabolism and excreted by the kidneys. Urea plays an important role in the medullary concentrating mechanism through a process called countercurrent exchange process (Weiner *et al.*, 2015). This process occurred through urea transporters (UTs). UTs are a channel like membrane proteins responsible for accumulation of urea in the renal inner medulla (Esteva-Font *et al.*, 2013). Two main UT subfamilies namely UT-A and UT-B have been identified in mammalians. The SLC14A2 gene p roduces several UT-A protein by alternative splicing. Two of these, UT-A1 and UT-A3, are

expressed in the inner medullary collecting duct (IMCD) and deliver urea in to the inner medullary interstitium from IMCD lumen. But, blood flow to the inner medulla would tend to dissipate the generated urea gradients. Dissipation, however, is returned by countercurrent exchange of urea through UT-A2 and UT-B expressed at the thin descending limbs of Henle and descending vasa recta, respectively. In addition, the UT-B urea channels present in erythrocyte membranes allow urea to exit rapidly from red cells and involve in this recycling process (Knepper & Miranda, 2013; Shayakul *et al.*, 2001).

UT inhibitors could be used as aquaretic agents to increase water excretion without changes in electrolyte excretion. Such compound would potentially be useful in treatment of hyponatremic disorders, including CHF, ascites, nephrotic syndrome and refractory edema (Knepper & Miranda, 2013). Triazolothienopyrimidines are an example of UT-B inhibitors that fully and reversibly inhibit urea transport (Yao *et al.*, 2012).

1.7. Medicinal plants as diuretics

Since the ancient time, mankind has been using plants as medicine. About 80% of world population uses herbal medicines for prevention and treatment of diseases, and the demand is increasing in both developed and developing countries (Tefera & Kim, 2019). Traditional medicinal plants are the integral part of culture of many African countries. About 3000 plant species are used by approximately 200,000 traditional healers in South Africa. It has also been used as a source of medicine to treat different ailments in East Africa, Kenya and other countries (Buwa-Komoren *et al.*, 2019; Jeruto *et al.*, 2008). In Ethiopia, there is a long history of using medicinal plants to treat a variety of ailments. Nearly 80% of the human population and 90% of livestock in Ethiopia rely on traditional medicine, as most plant species have very effective medicinal value for some ailments (Birhanu *et al.*, 2015). The major reasons why Ethiopia has relied on medicinal plants are culturally linked traditions, communities' trust on traditional medicine, and relatively low cost. Among numerous medicinal plants used in this country, *Ficus sur* is the one (Mesfin *et al.*, 2014).

Various plants are used as a subject to medical experiment from which the extracts can be used for management of certain ailments (Yadav, 2013). One of the traditional uses of herbal medicines is to induce diuresis. This is supported by a number of comprehensive reviews made on herbal medicines used as diuretics (Dutta *et al.*, 2014). For example, *Ficus glumosa* Del (Ntchapda *et al.*,

2014), *Carissa edulis (*Nedi *et al., 2004), Ajuga remota* Benth (Hailu & Engidawork, 2014), *Rumex abyssinicus* Jack (Getahun & Engidawork, 2015), *Moringa stenopetala (*Fekadu *et al., 2017), Moringa oleifera, Carum carvi and Tanacetum vulgare (*Lahlou *et al., 2007;* Tahkur *et al., 2016), Cissampelos pareira* L. Var, *Cyclea peltata* Thoms, *and Stephania japonica* Thunb (Hullatti *et al.*, 2011) are among many others.

1.8. The Experimental plant, *Ficus sur* Forssk

Ficus sur which is also known by other names.i.e. *Ficus capensis* Thunb, *Ficus mallotocarpa* Warb *and Ficus riparia (Miq. Ficus sur L.)* (Lansky & Paavilainen, 2010) belongs to the family of Moraceae and genus of Ficus. The genus *Ficus* consists of over 800 species such as *Ficus pandurate* Hance, *Ficus sycomorus*, *Ficus glomosa* and *Ficus exasperate* Vahl (Sirisha *et al.,* 2010). In Ethiopia, *Ficus sur* is known by different vernacular names as Shola in Amharic, Harbuu in Afan Oromo and Odakko in Sidamenya. It is also commonly known as wild fig, cape fig, broom cluster fig and bush fig in English (Beyi, 2018b; Regassa, 2013). This plant is widely distributed in South Africa, Cameroon, Ethiopia, Eritrea, Zaire, Cote D'Ivoire, Gambia, Ghana a nd Arabian Peninsula (Zachariades, 1994).

Ficus sur is a freestanding buttressed tree that grows up to a height of 25m along river banks, streams and forest margin. The bark is smooth, grey, darker grey-brown with age. The leaves are large, broadly oval, to 13 x 20 cm, usually smooth, edge sometimes wavy and often widely toothed, veins clear below, stalk grooved and flexible to 6 cm. The figs are in heavy clusters on branches to 70 cm long from trunk, orange-red, often hairy, soft and edible but watery and tasteless, having many seeds and often insects too (Germishuizen *et al.*) (Figure 2). Flavonoids, alkaloids, saponins, steroids, glycosides, terpenoids, tannins and hydrogen cyanide have been isolated and quantified from this plant (Achi *et al.*, 2017). Plants containing tannins can be used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumours while terpenoids possessing medicinal plants used as anticarcinogenic, antimalarial, anti-ulcer, antimicrobial and diuretic activity (Saxena *et al.*, 2013).

A) B)

C)

Figure 2: Photograph of *Ficus sur* Forsk: A) The tree, B) The leaves and C the figs from site of collection (captured on 27/04/019)

1.8.1. Traditional uses of *Ficus sur*

Ficus sur possess agro forestry and ecological importance. It is used as a source of food for humans and animals, as figs are edible, as well as for wood working. The leaves are also eaten as it was reported to help for blood boosting and anti-sickling activity of red blood cells (Adebayo *et al.*, 2017; Regassa, 2013). In addition to its economic importance, *Ficus sur* is traditionally used for

treatment of different diseases in different countries. In Sudan and Nigeria, its leaves and roots are used for treatment of leukoderma, leprosy, wounds, edema, respiratory disorders, diarrhea, sexually transmitted diseases, tuberculosis, anaemia, epilepsy, rickets, dysentery, male infertility and gonorrhea (Clark, 1996; Muhammad, 2017). A review of *Ficus sur's* ethnomedicinal uses also showed to treat swellings, edema, eye problems, toothache, general body pain, lung and sore throat and gonorrhea (Esievo *et al.*, 2018). In south Africa and other countries it is traditionally used in kidney problems, pain, malnutrition, parasitic infection, leprosy, paralysis, convulsions, spasm, gout, venereal diseases, and as diuretic (Lansky & Paavilainen, 2010; Mzindle, 2017). In Ethiopia, fresh leaves of the plant are pounded, mixed with water and given orally as a traditional medication for urine retention. There is also the traditional claim for the root part of this plant to be used for urinary problem (Beyi, 2018b; Regassa, 2013).

1.8. 2. Pharmacological activities

Apart from its traditional uses, *Ficus sur* extracts have been reported that it possessed anti-microbial activity (Solomon-Wisdom *et al.*, 2011), anti-sickling activity (Aja *et al.*, 2017), radical scavenging activity (Ramde-Tiendrebeogo *et al.*, 2012), gastrointestinal activity (antidiarrheal, spasmolytic) (Ayinde & Owolabi, 2009) anti-convulsant and sedative activities (Ishola *et al.*, 2013), anti-malarial activity (Muregi *et al.*, 2007), anti-inflammatory activity (Mzindle, 2017), anti-abortifacient activity (Njoku-Oji *et al.*, 2015) and for the treatment of erectile dysfunction (Akomolafe *et al.*, 2016; Muhammad, 2017). In other studies, it has been scientifically proven to be useful in obesity, diabetes, and dysentery (Ojokuku *et al.*, 2010). Moreover, several pharmacological investigations have indicated the plant to have activities, including against leprosy, rickets, infertility, gonorrhea, respiratory disorders, and as emollient (Awolola, 2015; Ramde-Tiendrebeogo *et al.*, 2012) as well as in diabetes (Chinonye *et al.*, 2014) among others. Plant of the same genus i.e. *Ficus glumosa* Del and *Ficus exasperate* Vahl have also been demonstrated for their diuretic activity (Amonkan *et al.*, 2013b; Ntchapda *et al.*, 2014).

1.9. **Rationale for the study**

Although most diuretics proved to be very effective in promoting sodium excretion, all could lead to potassium loss and provoked the search for potassium sparing diuretics. The issue of rebound Na^+ retention by loop diuretics also initiated the development of new agents with prolonged half-

life and/or extended release formulations. Furthermore, most plant species are very effective for some ailments with relatively low cost. Therefore, exploring for a new diuretic agent that maintains therapeutic efficacy and is devoid of potassium loss is justified. The number of plants have been documented that used traditionally as diuretic agents and some of them are confirmed for their claimed activities. *Ficus sur* is one of the plants found in our country and the fresh leaves of the plant are macerated to treat urinary retention traditionally as diuretic agent (Beyi, 2018a). But, its diuretic activity has not yet been validated experimentally. Accordingly, this study was aimed to evaluate the diuretic activity of aqueous and 80% methanol leaves extracts of *Ficus sur* in rats.

2. Objectives of the study

2.1. General objective

To evaluate the diuretic activity of aqueous and 80% methanol crude leaves extracts of *Ficus sur* Forssk in rats.

2.2. Specific objectives

- To assess acute toxicity of aqueous and 80% methanol leaves extracts of *Ficus sur*
- To evaluate the effect of aqueous and 80% methanol leaves extracts of *Ficus sur* on urine volume
- To determine the effect of aqueous and 80% methanol leaves extracts of *Ficus sur* on urinary electrolyte concentration
- To determine the effect of aqueous and 80% methanol leaves extracts on Urinary pH

3.9. Statistical analysis

Analyses were performed using international business machine of statistical package for the social Sciences, (IBM SPSS), version 25 for windows (SPSS inc, Chicago, Illinois, USA). Experimental results were expressed as mean ± SEM (standard error of mean) and statistical significance test was carried out by one-way analysis of variance (ANOVA) followed by the Tukey post hoc test to compare results among groups. $p < 0.05$ values were considered statistically significant.

3. Materials and methods

3.1. Chemicals and solvents

Chemicals and solvents used in this study include Absolute methanol (Lova Chemie, India), Distilled water (DW) (Social pharmacy and Pharmaceutics Laboratory, Addis Ababa University), Normal saline (Addis Pharmaceutical Factory, Ethiopia), furosemide (Epharm, Ethiopia).

3.2. Experimental animals

Healthy Wistar albino rats (200 – 245 gm) of either sex which reared in the animal house of the School of Pharmacy, Addis Ababa University, were used for the experiment. The animals were housed in plastic cages (6-8 rats per cage) under standard environmental conditions. The animals had access to rodent pellets and water *ad libitum*. Before they used for the experiment the animals were acclimatized to laboratory condition for a period of five days. The care and handling of animals were in accordance with internationally accepted guidelines for use of the experimental animals (Herling *et al.*, 1997; Vogel & Vogel, 2013) and the study was approved by the ethics committee of the School of Pharmacy, Addis Ababa University (ERB/SOP/213/03/2019).

3.3. Plant collection

The fresh leaves of *Ficus sur* were collected from Dugda district, Oromia Region, situated 134 km from Addis Ababa, Ethiopia on April/27/2019. Thereafter, taxonomic identification was done by Ato Melaku Wondafrash (MSc) and a voucher specimen MA/001 was given and deposited at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University for future reference.

3.4. Extraction of the plant

The leaves were cleaned from the dust and debris and washed gently with water; sliced to smaller pieces using scissors to reduce the size. The sliced pieces of the leaves were then pounded using a domestic food processor (onion grinder) and subjected to extraction.

3.4.1. Aqueous extraction

Five hundred grams of the pounded fresh leaves of *Ficus sur* were cold macerated in 1000ml of DW and allowed to stand at room temperature for 72 hours with occasional manual shaking. The macerate was first filtered using cotton gauze and later through Whatman filter paper No.1. Subsequently, the residue was remacerated twice in 500ml and 250 ml of DW using same procedure for a total of nine days. The filtrate was then freeze-dried with a Lyophilizer (OPR-FDU-5012, Korea) and semi-solid pasty mass of dark brown color was obtained. The dried aqueous extract was collected and the total yield was found to be 14.5 % (w/w). The dried extract was stored in temperature reading (-20^{o}c) until use and reconstituted with DW for oral administration.

3.4.2. Methanol extraction

Five hundred grams of the pounded fresh leaves of *Ficus sur* were cold macerated with about 1000 mL of 80% methanol and allowed to stand at room temperature for 72 hours with occasional manual shaking. The macerate was filtered using Whatman filter paper No. 1 and the residue was re-macerated twice for a total of nine days to extract the plant material exhaustively. The methanol was then evaporated from the extract under reduced pressure using Rota vapour (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 10.5% (w/w). The dried extract was stored in refrigerator until use and reconstituted with DW for oral administration.

3.5. Acute toxicity study

The acute oral toxicity test was performed on two groups, each consisting of five female rats, using the limit test recommendations of Organization for Economic Co-operation and Development (OECD) 425 Guideline (OECD, 2008). Healthy, and non-pregnant Wistar albino rats (age of 8-12 weeks) weighing from 200 – 245gm were employed for this test. The first group was treated by aqueous extract with dose of 2000 mg/kg (2 ml/100g) body weight whereas the second group received 80% methanol extract of the same dose orally, after being fasted for 18 hrs. On day one, a rat (from each group) was given an oral dose of 2000 mg/kg of the extracts in distilled water by oral gavage. Then rats were closely observed for behavioral or physical changes during the first 30 min (with special attention during the first 4 hrs.), thereafter for 24 h. On day two, other four

rats (from each group) were given the same dose and observed for the occurrence of manifestations for 14 days such as tremors, convulsions, salivation, diarrhea, weight loss, lethargy, sleep and coma.

3.6. Grouping and dosing of animals

Rats were randomly assigned into eight groups each consisting of six rats for diuretic test (Vogel & Vogel, 2013). Group-I was negative control treated with DW used for reconstitution. Group-II was positive control treated with 10 mg/kg furosemide. Group III-V were treated with doses of 100 mg/kg, 200 mg/kg and 400 mg/kg of *Ficus sur* aqueous extract respectively. Group VI-VII were treated with doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg of *Ficus sur* 80% methanol extract respectively. Dose selection was made based on the acute oral toxicity test performed, taking one tenth of the limit dose as the middle dose. Route of administration used for all groups was oral using gavage. The volume was calculated 2 mL/100gm by according to (OECD, 2008).

3.7. Determination of diuretic activity

Diuretic activity was determined following the method used by Lahlou *et al.*,(2007) with slight modification . Each rat was placed in an individual metabolic cage (Metabolic cage for rats, UGO BASILE, Italy) 24hrs prior to initiation of the experiment for adaptation and then fasted for 18 hrs with free access to water *ad libitum*. Before treatment, all animals were given oral saline in a volume of 15 mL/kg body weight (Nedi *et al.*, 2004). Each group then received the extracts, FURO10 and DW by oral gavages. Immediately after dosing, the rats were placed individually in a metabolic cage. The urine was then collected, measured and the pH determined at 1, 2, 3, 4, and 5 hr. Finally, the urine was stored at −20°C for electrolyte analysis.

The following parameters were calculated in order to compare the effects of extracts with those of the vehicle and standard. The urinary excretion, independent of the animal weight, was computed 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 one was used as a measure of diuretic action of a given dose of an agent (Formula −2). A parameter known as diuretic activity was also calculated as the ratio of diuretic action of the extracts in the test group to that of the standard drug (Formula – 3) (Kebamo *et al.*, 2015).

$$\text{Urinary excretion} = \frac{\text{Total urionary out put}}{\text{Total liquid administered}} \text{X}100$$

$$\text{Diuretic index} = \frac{\text{Urinary excretion of treatment groups}}{\text{Urinary excretion of control group}} X100$$

$$\text{Diuretic Activity} = \frac{\text{Diuretic index of test drug}}{\text{Diuretic index of standard drug}} X10$$

3.8. Analytical procedure

Urinary Na^+, K^+, and Cl^- concentrations of the plant extracts, control, and standard groups were analyzed using Ion Selective Electrode (ISE) analyzer (AVL 9181 Electrolyte Analyzer, Roche, Germany). The instrument was automatically calibrated prior to analysis with different standard solutions. The ratios of electrolytes Na^+/K^+ and $Cl^-/K^+ + Na^+$ were calculated to evaluate the natriuretic index and CA inhibition, respectively. In addition, urine pH was directly measured on fresh urine using a pH meter. Furthermore, the salt content of the extract was determined to rule out its effect on urinary electrolyte concentration.

4. Results

4.1. Acute toxicity test

From the acute toxicity test, no visible sign of toxicity was observed indicating that the median lethal oral dose of *Ficus sur* in rats was greater than 2000 mg/kg body weight.

4.2. Diuretic activity

4.2.1. Effects of the extracts at each time point

The highest dose (FSAE400) of aqueous extract produced significant diuresis ($p<0.05$) at the third hour. But, the 80% methanol extract did not show effect at all dose levels. The standard drug (FURO10) also produced apparent urine output at the same time point (Table 2).

Table 2: Effect of aqueous and 80% methanol extracts of the leaves of *Ficus sur* at each time point of 5hrs urine volume in rats

Group	Volume of urine (ml)					Diuretic action	Diuretic activity
	1hr	2hr	3hr	4hr	5hr		
DW/NC	1.83±0.32	2.53±0.42	0.43±0.21	0.62±0.31	0.52±0.23	1.00	
FURO10	2.75±0.36	1.87±0.40	3.15±0.74[a2]	1.67±0.44	0.67±0.33	1.52	1.00
FSAE100	1±0.51	1.85±0.38	2.33±0.24	1.88±0.38	1.27±0.32	1.26	0.83
FSAE200	1.63±0.39	2±0.25	2.33±0.34	1.92±0.15	1.38±0.17	1.39	0.91
FSAE400	1.77±0.18	2.38±0.14	2.43±0.24[a1]	2.07±0.23	1.57±0.35	1.47	0.97
FSME100	2.4±0.77	2.27±0.59	1.97±0.67	0.92±0.41	0.47±0.21	1.25	0.82
FSME200	2.55±0.31	2.38±0.31	1.93±0.29	0.92±0.43	0.42±0.15	1.27	0.83
FSME400	2.47±0.19	2.42±0.47	1.95±0.35	1±0.34	0.77±0.32	1.41	0.92

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; [1]$p < 0.05$, [2]$p < 0.01$; AE refers to aqueous extract

and ME to 80% methanol extract of *Ficus sur*; FURO10: Furosemide 10, DW/NC: controls treated with distilled water; Numbers refer dose in mg/kg.

4.2.2. Effect of the extracts on cumulative urine volume

The aqueous extract of *Ficus sur* produced dose dependent diuresis, albeit insignificant across the first three time points (Table 3). The aqueous extract showed significant urine output starting from the fourth hour with middle dose (45.3%, $p<0.05$) and higher dose (59.6%, $p<0.01$), which continued in the 5^{th} h, FASE200 (56.3%, $p<0.001$) and FASE400 (72.3%, $p<0.001$) compared to controls. Unlike the middle and higher dose of the extract, FASE100 produced apparent urine output at the last time point only (40.4%, $p<0.01$). Further inter group analysis also revealed that FSAE400 showed a significant difference in urine output at the fifth hour compared to FSAE100 (22.6%, $p<0.05$), FSME100 (27.4%, $p<0.01$) and FSME200 (24.6%, $p<0.05$). FURO10 treated rats exhibited a significant increment in urine volume setting in the third hour (61.8%, $p < 0.01$) and onwards. In addition, FURO10 displayed a significantly greater effect against FSAE100, FSME100 and FSME200 (23.44%, $p < 0.05$) at the fifth hour.

The middle dose (FSME200) displayed a detectable difference in urine volume as compared to the FSAE100 rats at the second hour (72.9%, $p<0.05$) (Table 3). But, no apparent difference was noted between controls and rats treated with all doses of methanol extract (FSME100, FSME200 and FSME400) until the third hour. FSME200 and FSME400 showed significant change at the fourth hour (43.5% & 44.5%, $p < 0.05$) and the fifth hour (38.2% & 45%, $p < 0.01$ & $p<0.001$), respectively, compared to the controls. On the contrary, FSME100 produced significant difference only at the fifth hour (35.24%, $p<0.05$).

Table 3: Effect of aqueous and 80% methanol extracts of the leaves of *Ficus sur* on 5 hrs cumulative urine volume in rats

Group	Volume of urine (ml)					Diuretic Index	Diuretic activity
	1hr	2hr	3hr	4hr	5hr		
DW/NC	1.83±0.32	4.37±0.22	4.80±0.30	5.42±0.35	5.93±0.30	1	
FURO10	2.75±0.36	4.62±0.54	7.77±0.97^{a2,c1}	9.43±0.80^{a3,c1}	10.10±0.60^{a3,c1,f1,g1}	1.52	1
FSAE100	1.00±0.52	2.85±0.59	5.18±0.50	7.07±0.68	8.33±0.50^{a2}	1.26	0.83
FSAE200	1.63±0.40	3.63±0.53	5.97±0.36	7.88±0.30^{a1}	9.27±0.37^{a3}	1.39	0.91
FSAE400	1.77±0.18	4.15±0.24	6.58±0.24	8.65±0.37^{a2}	10.22±0.17^{a3,c1,f2,g1}	1.47	0.97
FSME100	2.40±0.77	4.67±0.37	6.63±0.41	7.55±0.23	8.02±0.15^{a1}	1.25	0.82
FSME200	2.55±0.32	4.93±0.51^{c1}	6.87±0.46	7.78±0.33^{a1}	8.20±0.38^{a2}	1.27	0.83
FSME400	2.47±0.19	4.88±0.53	6.83±0.47	7.83±0.57^{a1}	8.60±0.42^{a3}	1.41	0.92

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 FSAE100, ^{f}against FSME100, ^{g}against FSME200; $^{1}p < 0.05$, $^{2}p < 0.01$, $^{3}p < 0.001$; FSAE refers to *Ficus sur* aqueous extract and FSME to 80% *Ficus sur* methanol extract; FURO10: Furosemide, DW/NC: controls treated with distilled water; Numbers refer dose in mg/kg.

4.3. Saluretic activity

4.3.1. Aqueous extract

The cumulative urine samples collected over five hours were analyzed for the electrolytes content (Na^+, K^+, and Cl^-) (Table 4). All the three doses of aqueous extract increased Na^+ excretion by at least 201%, ($p<0.001$) compared to NC group. Also all the three doses of aqueous extract significantly increased Cl^- excretion compared to NC rats. FURO10 also increased Na^+ loss by (542.5%, 113.3% and 147.3%, $p < 0.001$) compared to the NC, FSAE100 and FSME100, respectively. The Cl^- loss produced by FURO10 was significantly higher compared to NC and the lowest doses of both extracts. FURO10 significantly increased K^+ loss by (189.9%, $p<0.01$) and (81.1%, 105%, $p<0.05$) compared with the NC, FSAE400 and FSME100, respectively. Comparing

the extracts to each other, FSAE400 significantly increased Na^+ excretion by 78.30% and 106.7% compared to the FSAE100 and FSME100, respectively.

4.3.1. Methanol extract

All doses of the methanolic extract produced significant Na^+ excretion by (159.8%, $p<0.01$), 294.6% and 339.2% ($p < 0.001$) from low to high dose respectively compared to the control group (Table 4). The Cl^- excretion profile was increased by 150.3% with FSME200 and 163% with FSME400 ($p < 0.01$).

Table 4: Effect of aqueous and 80% methanol extracts of the leaves of *Ficus sur* on 5 hrs urinary electrolyte excretion in rats

Group	Urinary electrolyte concentration (mmol/L)			Saluretic index			Na^+/K^+	$Cl^-/ Na^+ + K^+$
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-		
DW/NC	15.33±2.38	27.68±5.80	20.73±3.43				0.55	0.48
FURO10	98.5±2.03^{a3,c3,f3}	80.27±10.51^{a2,e1,f1}	97.92±3.27^{a3,c3,f3}	6.42	2.90	4.72	1.22	0.54
FSAE100	46.17±5.12^{a3}	39.33±8.79	52.63±7.83^{a2}	3.01	1.42	2.54	1.17	0.61
FSAE200	55.67±4.63^{a3}	58.89±4.48	46.77±3.91^{a1}	3.63	2.13	2.26	0.95	0.41
FSAE400	82.33±5.61^{a3,c3,f3}	44.32±4.96	74.93±3.88^{a3,d1,f3}	5.37	1.60	3.61	1.86	0.59
FSME100	39.83±1.74^{a2}	39.14±2.97	33.52±2.71	2.60	1.41	1.62	1.02	0.42
FSME200	60.5±2.74^{a3,f2}	51.59±6.70	51.90±6.36^{a2}	3.95	1.86	2.50	1.17	0.46
FSME400	67.33±3.56^{a3,c2,f3}	62.58±13.33	55.38±9.36^{a2}	4.39	2.26	2.67	1.08	0.43

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, ^{c}against FSAE100, ^{d}against FSAE200, ^{e}against FSAE400, ^{f}against FSME100; $^1p < 0.05$, $^2p < 0.01$, $^3p < 0.001$; FSAE refers to *Ficus sur* aqueous extract and FSME to 80% *Ficus sur* methanol extract; FURO10: Furosemide, DW/NC: controls treated with distilled water; Numbers refer dose in mg/kg.

4.4. **Electrolyte content of the extract**

Water soluble salts that could present in the extract may interfere with the urinary excretion of electrolytes. The quantitative determination of aqueous extract revealed that there were very low

amounts of electrolytes. Whereas the same doses of 80% methanol extract showed non-detectable levels.

Group	electrolyte concentration of extracts (mmol/L)		
	Na+	K+	Cl-
FSAE100	7.69mmol/l	8.3mmol/l	10.4mmol/l
FSAE200	4.7mmol/l	6.9mmol/l	5.4mmol/l
FSAE400	3.4mmol/l	6mmol/l	7mmol/l

4.5. **Urinary pH**

Urinary pH measurement unveiled that the different treatment groups of both extracts produced relatively alkaline urine. The pH of aqueous extract treated rats' urine showed an increase from FSAE100 (7.70) to FSAE400 (8.10). On the other hand, treatment with 80% methanol extract increased pH from 7.60 (FSME100) to 8.00 (FSME400). The NC group produced the lowest pH and the standard group an intermediate pH (7.50) between vehicle and extract treated groups (Figure 3).

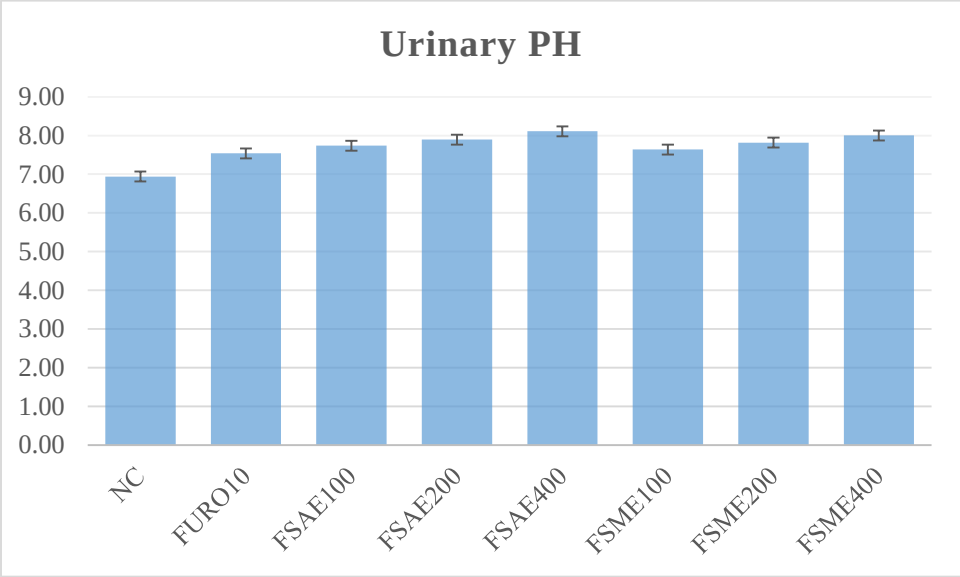

Figure 3:Urinary pH of rats treated with aqueous and 80% methanol extracts of the leaves of *Ficus sur*: FSAE refers to *Ficus sur* aqueous extract and FSME to 80% *Ficus sur* methanol extract; FURO10: Furosemide, DW/NC: controls treated with distilled water; Numbers

4.6. Onset of action of the extracts

The latency of first urination of the extracts in average was 27±3minets (Figure 4).

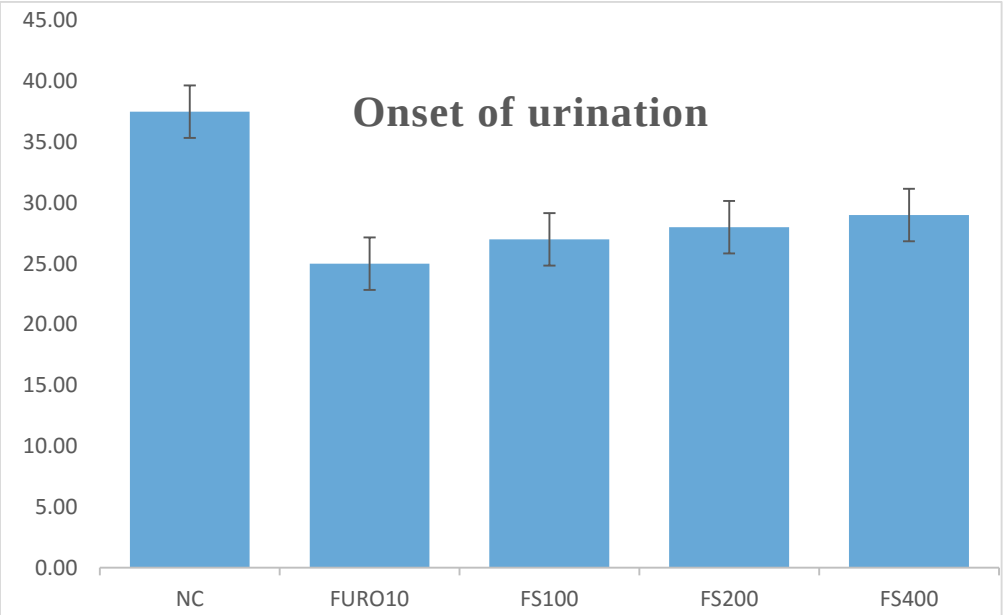

Figure 5: onset of urination of rats treated with aqueous and 80% methanol extracts of the leaves of *Ficus sur*: FSAE refers to *Ficus sur* aqueous extract and FSME to 80% *Ficus sur* methanol extract; FURO10: Furosemide, DW/NC: controls treated with distilled water; Numbers

5. Discussion

Diuresis could be beneficial for treatment of a number of disease conditions such as congestive heart failure, nephritic syndrome, hypertension, liver cirrhosis, poisoning, and certain kidney diseases. It is also important in kidney stone treatment. An increased fluid flowing through the kidney could dissolve the stones, flush out the deposits, and avoid further retention (Yuliana *et al.*, 2009). The results of this study revealed that *Ficus sur* had notable diuretic effects in the given animal model. As diuretics are employed clinically in treatment of edema, it would be most important to demonstrate effectiveness in the presence of electrolyte and water (Nedi *et al.*, 2004). Accordingly, saline was given to impose water and salt load or simulate edema.

A previous study done on acute and sub-chronic toxicity screening of chloroform extract of *Ficus sur* in rats revealed the median lethal concentration to be >5000 mg/kg body weight (Musa *et al.*, 2018). The present study was in line with this result suggesting that the leaves extracts of *Ficus sur* do not provoke toxic response in rat models.

Loop diuretics like furosemide increase urinary flow rate and urinary excretion of sodium, potassium and chloride by inhibiting Na^+–K^+–$2Cl^-$ symporter in the thick ascending loop and by inhibiting carbonic anhydrase enzyme (Sarafidis *et al.*, 2010a). Furosemide produced its maximum diuretic effect at a dose of 10 mg/kg, in agreement with values given in the literature, typical of saluretic diuretics type (Al-Saikhan & Ansari, 2016). The aqueous and 80% methanol extracts of the leaves of *Ficus sur* revealed an increase in cumulative urine volume that appeared to vary with dose and nature of the extract. The aqueous extract produced a better diuretic effect compared to the 80% methanol extract, particularly with increasing dose, FSAE400 vs. FSME100 (27.4%, $p<0.01$) and FSME200 (24.6%, $p<0.05$). Therefore, it is possible to suggest that the secondary metabolites responsible for increasing urine output could be polar and hence it is better to extract with water than 80% methanol. The lower doses of both extracts produced a noticeable effect only at the fifth hour whereas the medium dose of the aqueous extract produced significant effect at the fourth and fifth hours compared to negative control. Other than the cumulative diuretic effect of the plant extract, only the highest dose of aqueous extract produced significant diuresis at the third hour, showing the dose dependent effect of the plant. This may also suggest that the maximal effect of the aqueous extract may reach at this time point.

The diuretic activity is considered to be good if the diuretic index values are greater than 1.50, moderate if the values are in between 1.00 and 1.50, mild if the values lie in between 0.72 and 1.00 and nil if the value is < 0.72 (Asif *et al.*, 2014). Both extracts accordingly, at all doses, have shown a moderate diuretic activity as evidenced by the diuretic index values. The aqueous and 80% methanol extracts at 400mg/kg displayed a diuretic activity of 97% and 92%, respectively, compared to the reference drug, furosemide (10 mg/kg). This result conforms with the previous works reported from the same genus, *Ficus exasperata* Vahl, (Amonkan *et al.*, 2013a) suggesting that the largest doses of both extracts of *Ficus sur* produced similar diuresis and saluretic (Na^+ & Cl^-) excretion profile to that of furosemide.

Quantitative determinations of potassium ion present in the aqueous extract of *Ficus sur* showed very low concentration. This suggests that diuretic effect of the plant does not seem to be an osmotic type. The results are in agreement with other plant extracts reported elsewhere (Martín-Herrera *et al.*, 2007). On the contrary, the level of potassium ion in 80% methanol extract was non-detectable suggesting water soluble salts may not be present in sufficient amount. Thus, the diuretic effect of the plant extract was not due to its content of potassium salt rather due to its intrinsic ability of the plant.

The retention of sodium and increased water volume have long been considered key players in the pathogenesis of hypertension as well as edematous conditions such as cardiac failure and cirrhosis (Schrier, 2006). The effect of the extracts on urinary electrolyte concentration was clearly accompanied by sodium excretion which reinforces the diuretic effect of *Ficus sur* was of the saluretic type, in contrast to aquaretic type, a typical feature of most diuretics of plant origin (Martín-Herrera *et al.*, 2007). In addition, this saluretic diuretics activity of *Ficus sur* was similar with the result observed on *Ficus glumosa (Ntchapda et al., 2014)*. Based on the finding, the diuretic action of the plant might be through inhibition of Na^+-K^+-$2Cl^-$ cotransporter 2. It also was observed that both extracts exhibited a weak excretion of potassium which is less than the standard suggesting that it has potassium sparing diuretic activity as well.

The Na^+/K^+ ratio is a biomarker of mineralocorticoid receptor (MR) antagonism (Eudy *et al.*, 2011). The ratio of $Na^+/K^+ > 2$ shows the ability of the test substance to excrete a greater proportion of Na^+ in contrast to K^+ which is a very essential indicator for a good natriuretic and diuretic activities (Kumar *et al.*, 2010). The aqueous extract of 400mg/kg had a good natriuretic activity

(i.e. 1.86), preventing hypokalemia, a common problem associated with furosemide and hence could have a beneficial effect in different edematous conditions. Na^+/K^+ values >10 indicate a K^+-sparing activity which was not the case for both the experimental plant and furosemide in this study (Papademetriou, 2006). So, the extract could be compared with the sparing diuretic to confirm the rescue ability of potassium loss.

The $Cl^-/ (Na^+ + K^+)$ is indicative of carbonic anhydrase inhibitory activity, and substances having a value between 0.8 and 1.0 could be excluded. With decreasing ratios, slight to strong carbonic anhydrase inhibition can be assumed (Kebamo *et al.*, 2015). The present study, therefore, indicates that *Ficus sur* may have carbonic anhydrase inhibitory activity in renal tubules. The extracts also exhibited a relative increase in urine pH, suggesting that carbonic anhydrase inhibition to be another possible mechanisms of diuretic action. This alkalinization of urine also give a clue that the plant has potassium sparing diuretic activity. It also coincides with the results reported from the same genus, *Ficus glumosa* (Ntchapda *et al.*, 2014).

A previous study done on diuretic effect of *Ficus exasperata* showed that urinary excretion volume obtained after 6-24 hours was highest than that obtained after administration of furosemide (Amonkan *et al.*, 2013b). The current study showed that onset of the diuretic action of the extracts was sufficiently rapid as the latency of the first urination of the extracts was 27±3 minute and had a fairly long duration of action as it had effect from the first to the fifth hour of the experiment. This may be an important diuretic profile to shorten the frequency of administration.

Ficus sur was studied for the presence of a variety of secondary metabolites. In a previous study, the quantitative phytochemical analysis carried out on aqueous extract of *Ficus sur* leaves showed presence of flavonoids, terpenoids, alkaloids, and tannins in large amount; whilst saponins, steroids, glycosides and hydrogen cyanide are in trace amount (Achi *et al.*, 2017). On the other hand, phytochemical screening done on methanol extract of this plant revealed the presence of tannins, alkaloids, flavonoids, phenols, cardenolides, steroids and anthraquinones (Odusanmi, 2017; Solomon-Wisdom *et al.*, 2011). Furthermore, several studies have explained that flavonoids, saponins, terpenoids, tannins, alkaloids and organic acids were responsible for the diuretic activity of a plant extract (Saxena *et al.*, 2013; Yuliana *et al.*, 2009). For example, flavonoids were known to be A_1R antagonists (Moro *et al.*, 1998). Thus, the diuretic effect of this plant extract may be due to stimulation of regional blood flow which suggests that the identified compounds from

Ficucs sur is responsible for the observed diuretic activity. The compounds may act individually or synergistically.

6. Conclusion

The present study revealed both aqueous and methanol extracts of *Ficus sur* possessed diuretic and natriuretic activities. The present finding, therefore, supports the traditional claim of the plant as a diuretic agent. The urinary PH and electrolytes analysis results hint that the extracts could have multiple modes of action. The safety profile of the extract was an added advantage that calls for conducting further research to ascertain the findings reported in this study.

7. Recommendation

- ✓ Since diuretics are indicated for multiple chronic conditions chronic toxicity study should be done
- ✓ Since the plant extracts had a fairly long duration of action and a promising potassium conserving ability, they require further investigation for 24-hour urine output and using potassium sparing diuretics as a standard.
- ✓ The active principles responsible for the diuretic effects of the extracts of this plant have not yet been known. Thus, this finding advocates that there should be a need for further fractionation and isolation of pure secondary metabolite responsible for the diuretic activity.
- ✓ Further investigation is required in order to understand the precise mechanism of diuretic action of the extracts.

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