Elite Journal of Medicine. Volume 2 issue 5(2024), Pp. 16-27
https://epjournals.com/journals/EJM

Diuretic Activity of Ethanolic Root Extract and Fractions of *Agave Sasilana* in Albino Rats

*O. D. Omodamiro [1], R.M. Omodamiro [2] and Emmanuel Ifeanyi Obeagu[3]

[1] Department of Pharmacology and Toxicology, School of Pharmacy, Kampala International University, Uganda.
[2] Department of Pharmaceutical Chemistry and Quality Control, School of Pharmacy Kampala International University, Uganda.
[3]Department of Medical Laboratory Science, Kampala International University, Uganda.

[*]**Correspondence**: omodamiro@kiu.ac.ug

Abstract
The present study investigates the diuretic activity of the ethanolic root extract and fractions of *Agave sasilana* in albino rats. Forty-eight male albino rats of mean weight 180 g were used for the study. There were seven test groups and a control group. Regular doses of 25, 50, 75, 100, 125, 150 and 175 mg/kg body weight were administered intraperitoneally twice daily for 7 days to the rats in the treatment groups 1, 2, 3, 4, 5, 6 and 7 respectively. Animals in the normal control group were given 25 ml/kg body weight of normal saline. The same procedure was carried out for the reference group that received furosemide (25, 50, 75, 100, 125, 150, 175 mg/kg) body weight. The urine volume and electrolyte concentrations were measured to evaluate the diuretic activity of ethanolic root extract and fractions of *Agave sasilana.* The findings demonstrated that the crude extract and fractions showed significant diuretic, kaliuretic and natriuretic effects in the treated groups in a dose dependent manner when compared to the normal control. The diuretic index values showed good diuretic activity of the crude extract and the fractions. The crude extract and fractions of *Agave sasilana* increases the urine volume and concentration of urinary electrolytes in a dose-dependent manner. Therefore, this plant has a diuretic potential. However, future studies should focus on isolating the phytochemical component(s) responsible for diuresis.

Keywords*: Agave sasilana; diuretic activity; electrolyte; furosemide; urinary volume.*

1. Introduction
Substances that elevate the rate of urine flow and salt loss are known as diuretics [1-2]. The net excretory effect of diuretic agents causes changes in urine flow, pH, and ionic compositions of urine and blood [3]. Diuretic agents are important to promote a net loss of excessive accumulated body fluids, salts, toxemias, and other accumulated metabolic products including urea [4]. Diuretics mostly work by stimulating urine output together with urinary excretion of sodium from the body. Diuretics such as the high-ceiling loop and thiazides diuretic have been associated with several side effects, such as electrolyte and metabolic changes, new-onset diabetes development, renin-angiotensin system activation and weakening of sexual function [3]. This fact necessitates that there is a strong need for novel diuretics which are relatively safe with better or equivalent diuretic activity. However, the currently available diuretic agents are associated with numerous side effects

and diuretic resistance in some patients. For instance, the recent global cohort studies indicated that the prevalence of diuretic resistance was estimated to be 20–35% in heart failure cases [5, 6].
Since ancient times to this date, medicinal plants have been commonly used as a source of treatment for human disorders. Particularly in developing countries, a majority of people depend on herbal medicines to treat various illnesses [7]. Diuretic effect is one of the fields of application for botanicals, and herbal medicines are used to treat edematous disorders, such as heart failure, cirrhosis, and nephritic syndrome, that contribute to body-fluid overload [8].
Agave belongs to the family Agavaceae and is widely distributed in tropical and subtropical regions of the world, and, due to their ability to grow in dry lands and their several potential applications, plants of this genus have been called "plants of the century" [9]. In several countries, the juice of *Agave sisalana* leaves presents great ethnopharmacological importance because it is used as an antiseptic in the topical treatment of skin diseases as well as a poultice on wounds [10]. Orally, it is used to treat indigestion, flatulence, jaundice, constipation, and dysentery [11].
The widespread use of this medicinal plant by local people entails the necessity of testing of their efficacy and safety profile [12]. Therefore, this study aimed to investigate the effect of both the ethanolic root extract and different fractions of *Agave sasilana* on the diuretic activity in albino rats.

2. Materials and Method

Chemicals and Reagents

Chemicals and solvents used in this study were absolute ethanol (Lova Chemie, India), distilled water (Social Pharmacy and Pharmaceutics Laboratory, Addis Ababa University), normal saline (Addis Pharmaceutical Factory, Ethiopia), and furosemide (Epharm, Ethiopia). All chemicals used were of analytical grade.

Plant material

Fresh root part of the plant *Agave sasilana* were locally sourced in Umudike farm, Abia State, Nigeria and was identified by Prof. Garuba Omosun, a taxonomist, of the Plant Science and Biotechnology Department, Michael Okpara University of Agriculture, Umudike, Nigeria. The fresh roots were washed and dried under shade at room temperature, using a blender; the roots were blended into powder.

Extraction

The powdered roots of *Agave sasilana* (200 g) were soaked in ethanol for 48 hours and the extract filtered using a Whatman no. 1 filter paper, the filtrate was allowed to evaporate to dryness, under a water bath with a temperature set at 40°C.

Animals

Healthy looking male albino rats of mean weight of 180 g obtained from the the Veterinary College, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria were used for the study. All animals were kept in metabolic cages in the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria), under normal room conditions and acclimatized for two (2) weeks. Commercial pellet diet (Vital growers mash by Grand Cereals and Oil Mills, Nigeria) and water were given to the animals *ad libitum.*

Experimental design

Forty-eight (48) male albino rats of mean weight 180 g were used for the study. The animals for the study were grouped into eight groups of six rats each. There were seven test groups and a control group. Regular doses of 25, 50, 75, 100, 125, 150 and 175 mg/kg body weight were administered intraperitoneally twice daily for 7 days to the rats in the treatment groups 1, 2, 3, 4, 5, 6 and 7 respectively. Animals in the normal control group were given 25 ml/kg body weight of normal saline. At the end of the experiment, all the animals in the various groups were anaesthetized, dissected and blood collected via cardiac puncture. The blood samples were collected with EDTA sample bottle for the analysis of the diuretic activity of the ethanolic root extract and fractions of *Agave sasilana* in albino rats.

Determination of Diuretic Activity of Ethanolic Root Extract and Fractions of *Agave Sasilana*

The method of [13] was employed for the assessment of diuretic activity. In this method, male albino rats of mean weight 180 g deprived of food and water for 18 hours prior to the experiment were divided into eight groups of six rats in each group. The first group serves as control, received normal saline (25 ml/kg) other groups received doses of the extract at 25, 50, 75, 100, 125, 150, 175 mg/kg body weight. The same procedure was carried out for furosemide (25, 50, 75, 100, 125, 150, 175 mg/kg) body weight.

Immediately after the administration, the animals were place in metabolic cages (2 per cages), specially designed to separate urine and faeces kept at $20^{oc}\pm0.5^{oc}$. The volume of urine collected was measured at the end of 5 hours. During the period no food and water was made available to the animals. The parameter taken were total urine volume, concentration of Na^+, K^+, Cl^-, and HCO_3^- in the urine. Na^+ and K^+ concentration was determined by digital flame photometer. Cl^- and HCO_3^- concentration were estimated by literation with silver nitrate solution. Using 3 drops of 5% potassium chromate solution as indicator [14].

3. Results

The result of the diuretic activity of the ethanolic root extract of *Agave sasilana* is shown in figure 3.1. The volume of the urine produced increases with increase in the concentration of the extract. The ethanolic root extract of *Agave sasilana* exhibited high diuretic activity. The hydrogen carbonate concentration did not change significantly. The result of diuretic activity of standard drug (furosemide) is shown in figure 3.3. The urine volume produced increased with increased in doses (concentration) of furosemide. The result of diuretic activity of furosemide is shown in figure 3.4. The inhibition of sodium, potassium and chloride re-absorption by furosemide is concentration dependent. However, hydrogen bicarbonate re-absorption was not inhibited significantly.

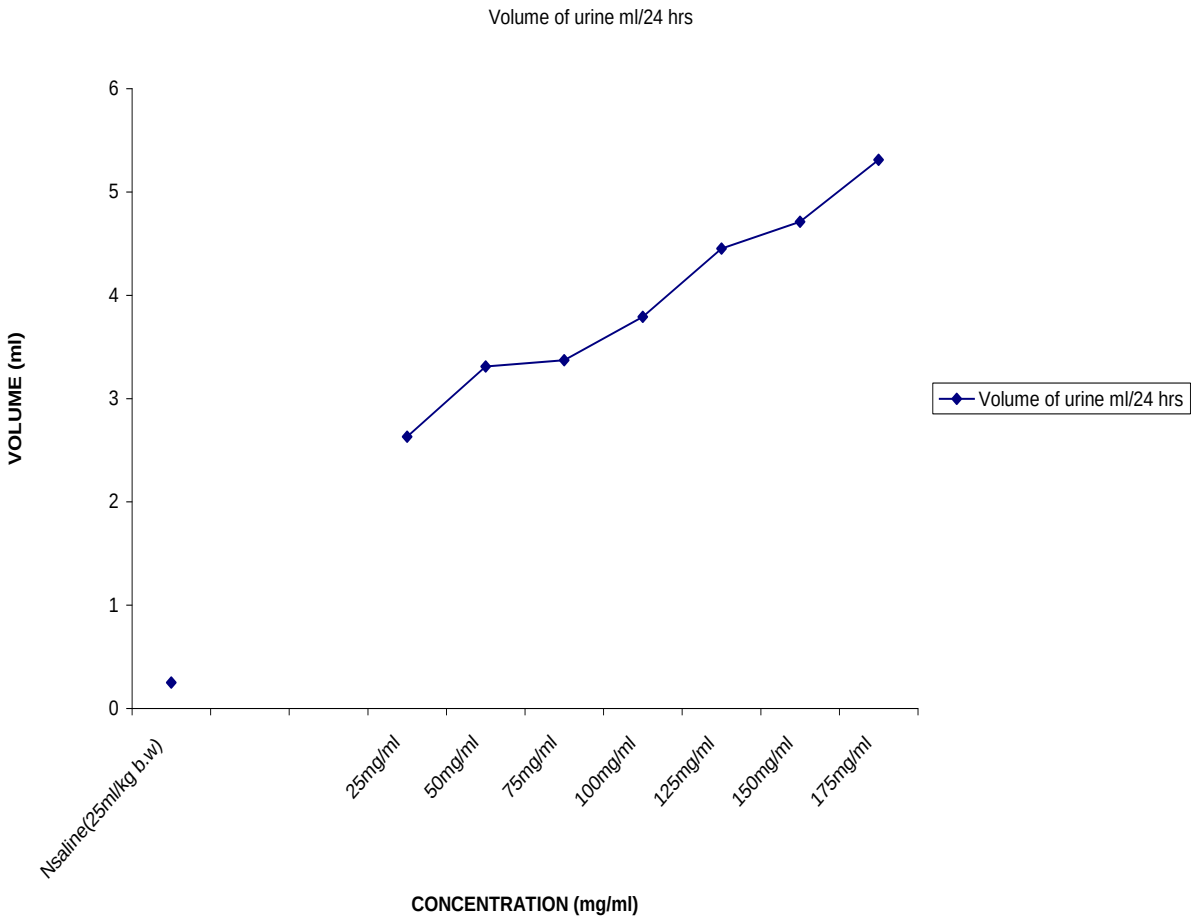

FIGURE 3.1: DIURETIC ACTIVITY OF THE CRUDE EXTRACT

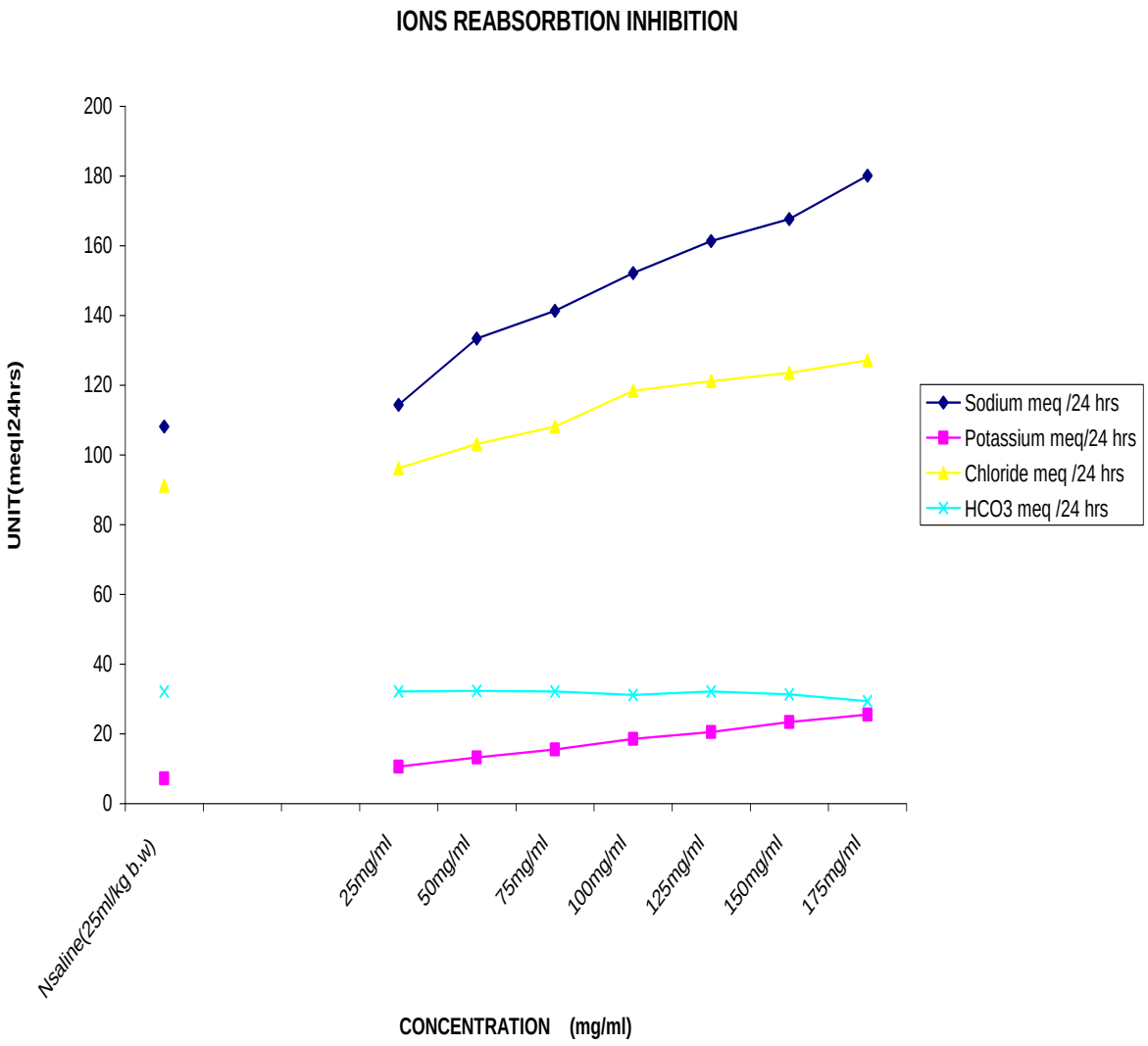

FIGURE 3.2: DIURETIC ACTIVITY OF CRUDE EXTRACT

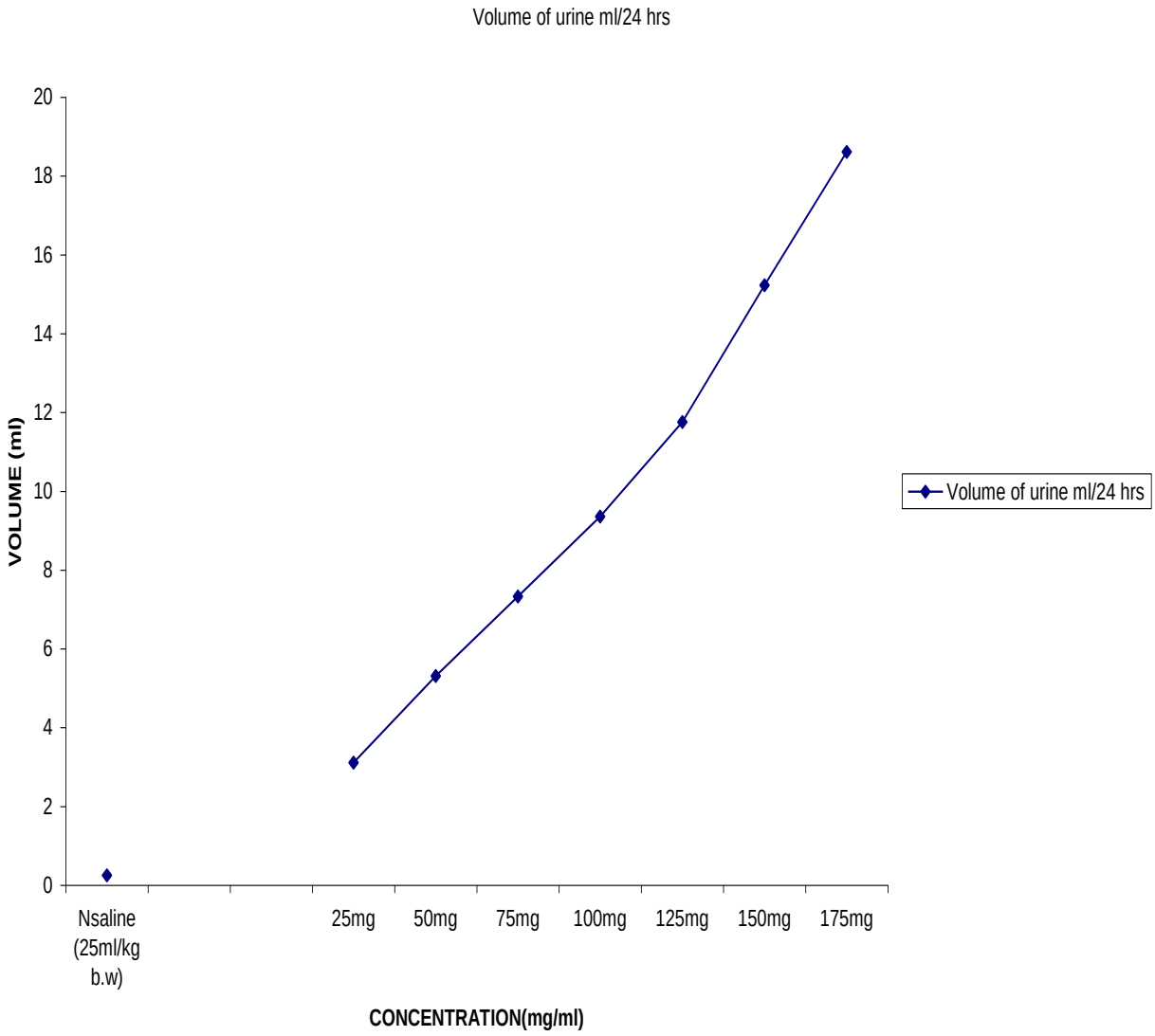

FIGURE 3.3: DIURETIC ACTIVITY OF FUROSEMIDE

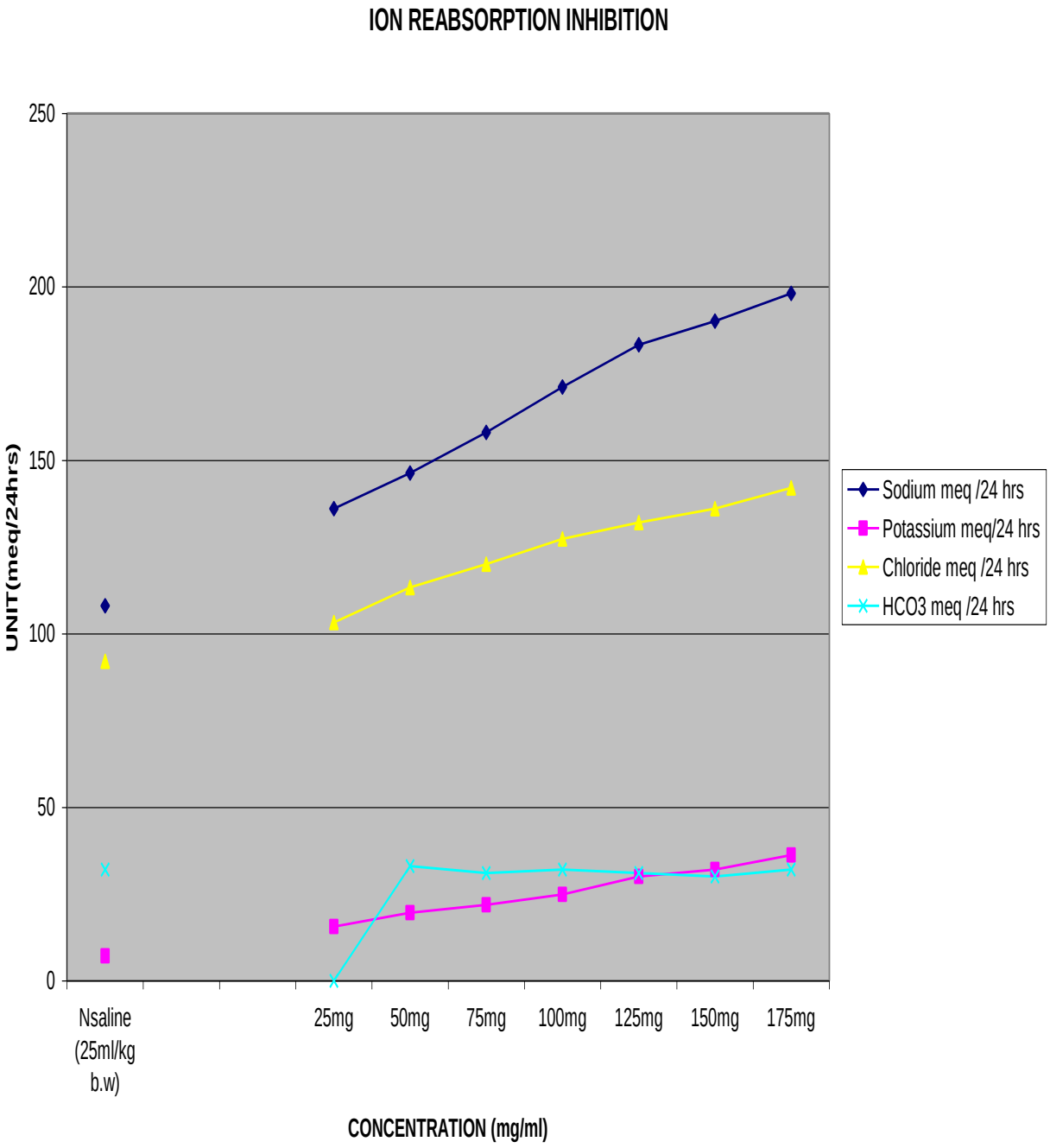

FIGURE 3.4: DIURETIC ACTIVITY OF FUROSEMIDE

The biological activities of the five fractions with respect to diuretic activity.

Fractions A to E contains flavonoids, alkaloids, saponins, tannins and anthraquinone which produced significant diuretic activity when compared to the control group.

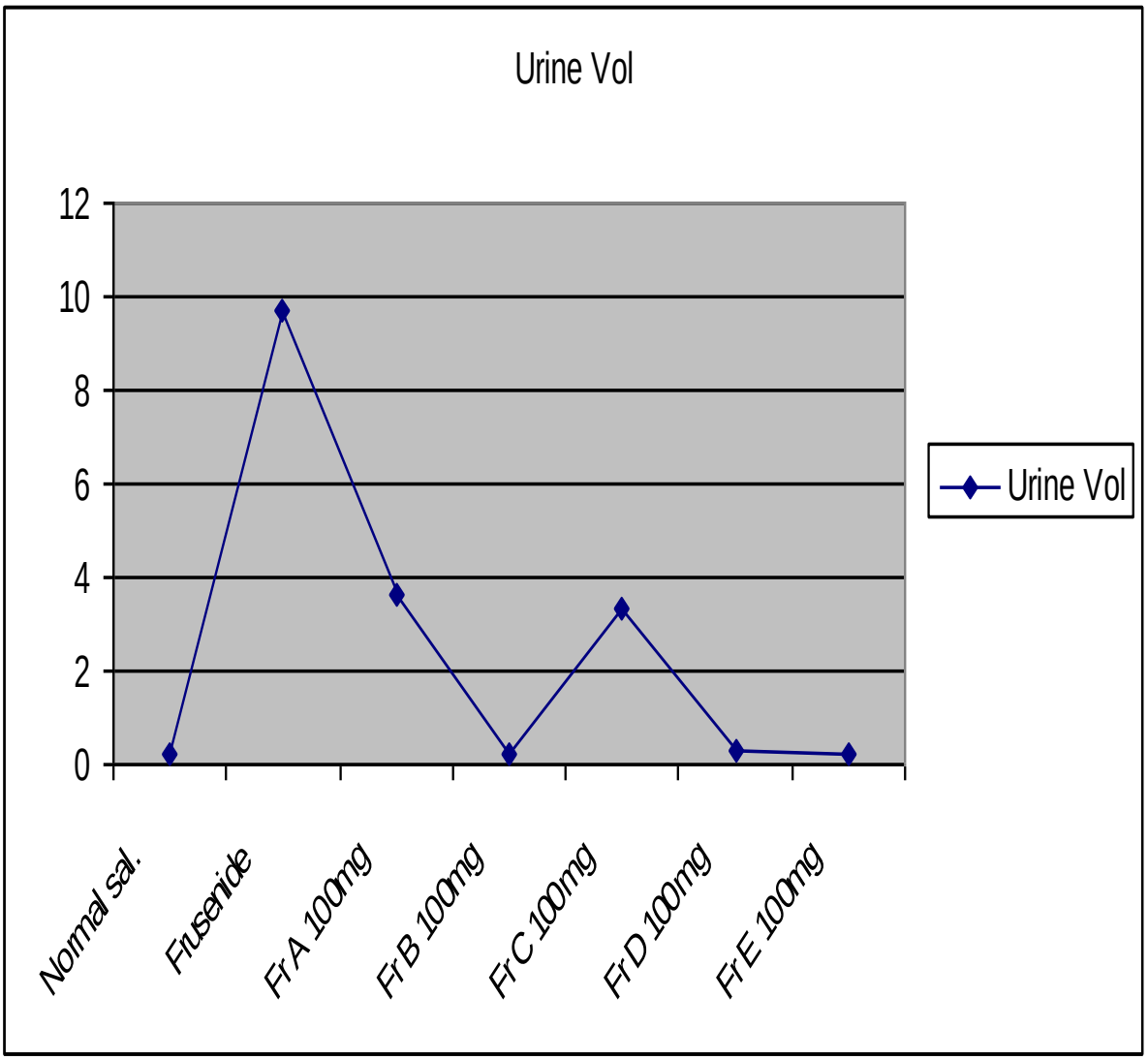

FIGURE 3.5: **DIURETIC ACTIVIY OF THE FIVE FRACTIONS OF *Agave sasilana* ETHANOLIC ROOT EXTRACT**

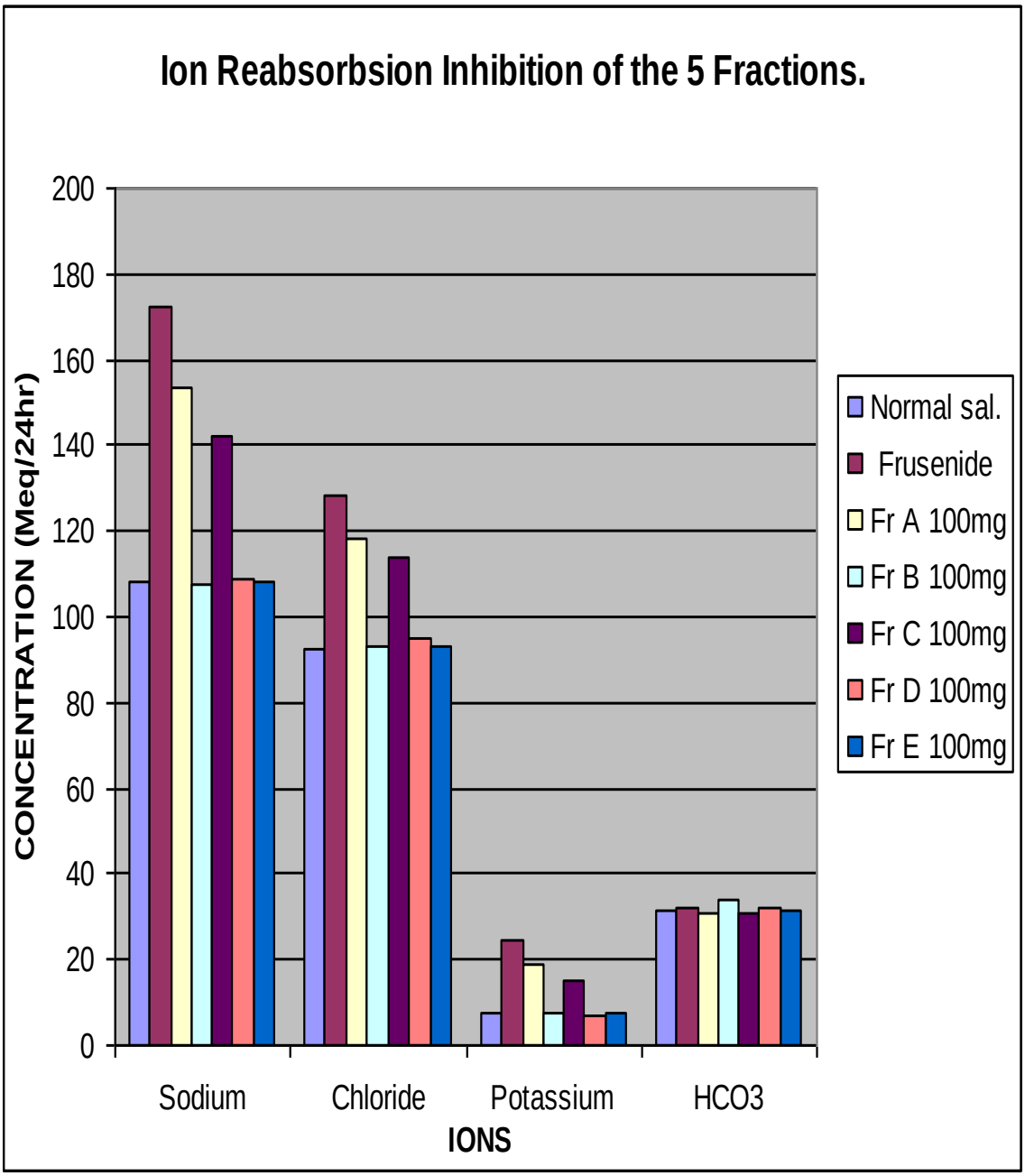

FIGURE 3.6: Diuretic Activity of The Five Fractions of *Agave Sasilana* Ethanolic Root Extract

4. Discussion

Diuretics are mainly used to adjust the volume and composition of body fluids in a variety of disorders, including hypertension, congestive heart failure, hepatic cirrhosis, and nephrotic syndrome [15.16]. This adjustment is mainly achieved through inhibition of reabsorption of water and electrolytes across tubular epithelial cells into the bloodstream [17]. In this study, urine volume and electrolyte concentrations were measured to evaluate the diuretic activity of ethanolic root

extract and fractions of *Agave sasilana.* Several medicinal plants have been proven to improve conditions of volume overload resulting from retention of electrolytes and water, with better safety profiles [18,19]. Therefore, it is very necessary to demonstrate effectiveness of plant extracts in the presence of electrolytes and water, [20] and so normal saline was loaded to simulate edema.

The present study reports the aquaretic and pronounced kaliuretic effects of ethanolic root extract and fractions of *Agave sasilana*. With regard to urine output, the extracts and fractions resulted in an increase in urine excretion in a dose-dependent manner. The effect turned out to be more significant at higher doses compared to the control group, possibly due to increased concentration of active components. The small increase in diuresis observed with the ethanolic root extract and fractions of *Agave sasilana* could possibly be explained by polar ingredients in the plant material, which may be responsible for increasing urine output. However, the extracts at lower doses did not produce any appreciable effect, probably owing to insufficient active components responsible for induction of diuresis [20].

In view of electrolyte composition of urine, the higher dose of the extract produced significant increases in urinary excretion of all ions compared to the control group, Urinary excretion of Na^+, K^+, and Cl^- was not elevated significantly at the lower doses of the extract. The excessive K^+ excretion observed in this study might have been due to high K^+ concentration in the extracts [21]. Increased Na^+:K^+ implies more Na^+ excretion than K^+, which is regarded a very good profile for diuretic agents. However, neither the crude extract nor the fractions increased the Na^+:K^+ ratio, indicating that the plant has low natriuretic potential but a pronounced kaliuretic effect. The observed K^+-wasting effects of the extracts may not be enough to comment on whether the plant has a potassium-sparing effect or not, since when there is an increase in potassium intake, there will be an increase in its excretion as well [22]. When potassium overloading occurs, the kidney tubules are unable to absorb it and consequently produce urinary excretion of the osmotic type, which promotes water diuresis [23].

With regard to the patterns of urine output and excretion of electrolytes (K^+, Na^+, and Cl^-), together with the diverse bioactive principles present in the crude extract and fractions, it appears that the plant may have mechanisms of action similar to some herbal medications suggested to have a wide range of diuretic mechanisms [24,25]. As such, in addition to the suggested carbonic anhydrase–inhibitory activity, osmosis-like effects and increased glomerular filtration rate might be another mode of action that contributes to the diuretic effect of *Agave sasilana*.

5. Conclusion

Collectively, the results of this study revealed that both the ethanolic root extract and fractions of *Agave sasilana* possessed significant diuretic activity. Specifically, the larger doses of the extract produced notable diuresis. Urinary volume and electrolyte analysis showed that the extracts have many modes of action. This study thus substantiates this plant's traditional claim as a diuretic agent.

Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropiate ethics committe.

Acknowldgement
We sincerely appreciate all that made this work successful.

Competing Interests
Authors have declared that no competing interests exist.

References
1. Bharat VR. "Comparative diuretic study of medicinal plants in individual and combination form," Magazine. Pharmatutor. Org, 2017; 5(4):42–45.
2. Husain A, Madhesia D, Rashid DM, Ahmad A, Khan SA, "Synthesis and in vivo diuretic activity of some new benzothiazole sulfonamides containing quinoxaline ring system," Journal of Enzyme Inhibition and Medicinal Chemistry, 2016; 31(6):1682–1689.
3. Ellison DH. "Clinical Pharmacology in diuretic use," Clinical Journal of the American Society of Nephrology, 2019; 14:1–10.
4. Wile D. "Diuretics: a review," Annals of Clinical Biochemistry, 2012; 49(5):419–431.
5. Trullas J, Casado JL, Morales R. "Prevalence and outcome of diuretic resistance in heart failure," Internal and Emergency Medicine, 2009; 14(4): 529–537.
6. Carrizales-Sepulveda EF, Vera-Pineda R, Jimenez-Castillo RA, Benavides-Gonzalez MA, Ordaz-Farias A, "Prevalence and outcome of diuretic resistance in heart failure: comment," Internal and Emergency Medicine, 2019; 14(4):631-632.
7. Zhang X, Organization WH. Traditional medicine strategy 2002 2005; 2002.
8. Vazir A, Cowie MR. The use of diuretics in acute heart failure: evidence-based therapy? 2013.
9. Sidana J, Singh B, Sharma OP. Phytochemistry Saponins of Agave: Chemistry and bioactivity. Phytochemistry, 2016; 130:22–46.
10. Barreto SG, Maia MS, Benicá AM, Assis HS, Leite-Silva VR., Rocha-Filho PA, Negreiros MF, Oliveira HA, Ostrosky EA, Lopes PS. Evaluation of in vitro and in vivo safety of the by-product of Agave sisalana as a new cosmetic raw material: Development and clinical evaluation of a nanoemulsion to improve skin moisturizing. Ind. Crops Prod, 2017; 108:470– 479.
11. Zhang X, Liu L, Lin C. Structural features, antioxidant and immunological activity of a new polysaccharide (SP1) from sisal residue. Int. J. Biol. Macromol, 2013; 59:184–191.
12. Mba OJ, Aloh GS, Nwachukwu KC, Michael PO. Phytochemical Characterization, Acute Toxicity Studies of the Methanol Extract of *Napoleonae imperialis* Leaves. Journal of Genetics and Cell Biology, 2020; 3(3):194-198.
13. Hailu W, Engidawork E. Evaluation of the diuretic activity of the aqueous and 80% methanol extracts of Ajuga remota Benth (Lamiaceae) leaves in mice. BMC Compl Altern Med, 2014;14(1):135.
14. Lahlou S, Tahraoui A, Israili Z, Lyoussi B. Diuretic activity of the aqueous extracts of Carum carvi and Tanacetum vulgare in normal rats. *Jour Ethnopharmacol*, 2007;110(3):458–463.
15. Roush GC, Kaur R, Ernst ME. Diuretics: a review and update. Jour Cardiovasc Pharmacol Ther, 2014;19(1):5–13.
16. Hullatti K, Manjunatha J, Kuppasth I. Comparative Study on Diuretic Effect of Buchanania angustifolia Roxb., and Buchanania lanzan Spreng. Fruit Extracts and Fractions. J Appl Pharm Sci, 2014;4(8):59.
17. Wright C, Van-buren L, Kroner C, Koning M. Herbal medicines as diuretics: a review of the

scientific evidence. *Jour Ethnopharmacol*, 2007;114(1):1–31.
18. Ahmad W, Zeenat F, Ahmad M, Ansari N. Medicinal plants as potent diuretic: a review. *Int* Jour Adv Pharm Med Bioallied Sci. 2017;2017(2017):1–8.
19. Dutta KN, Chetia P, Lahkar S, Das S. Herbal plants used as diuretics: a comprehensive review. Jour Pharm Chem Biol Sci, 2014;2(1):27–32.
20. Nedi T, Mekonnen N, Urga K. Diuretic effect of the crude extracts of Carissa edulis in rats. *Jour Ethnopharmacol*. 2004;95(1):57–61.
21. Krishnakanth K, Kumar P, Neeraja K, Cheekavolu C. Effect of Sesbania grandiflora Linn leaf extracts on diuresis in wistar rats. Int J Basic Clin Pharmacol. 2017;6(6):1305.
22. Haji H, Makonnen E, Debella A, Geleta B. Evaluation of diuretic and antihypertensive activity of leaf extracts of Thymus schimperi in rats. British J Pharmacol Toxicol. 2016;7(1):1–8.
23. Ramin S, David P, E H. *Diuretic Agents Katzung*, Basic and Clinical Pharmacology. 13th ed. New York: McGraw-Hill Education; 2015: pp. 249–69.
24. Jayakody J, Ratnasooriya W, Fernando W, Weerasekera K. Diuretic activity of leaves extract of hot water infusion of ruta graveolens L. in rats. JPT. 2011;6(5):525–532.
25. Hullatti K, Gopikrishna U, Kuppast I. Phytochemical investigation and diuretic activity of Cyclea peltata leaf extracts. J Adv Pharm Technol Res. 2011;2(4):241.