



Diuretic Activity of the Hydroalcoholic Extracts of Rhizomes and Leaves of *Artemisia Abyssinica* Sch. Bip. ex A. Rich: *In Silico* and *In Vivo* Study

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Abstract

Background/Aim: The majority of communities in developing nations utilise traditional medicine as an alternative or a combination therapy with a clinically approved diuretic regimen. The present study aimed to investigate the *in vivo* and *in silico* diuretic properties of the 80 % methanol extracts of the rhizomes and leaves of *Artemisia abyssinica*, an indigenous traditional diuretic medicinal plant of Ethiopia.

Methods: Acute oral toxicity tests of 80 % methanol rhizome and leaf extracts of the plant were conducted in mice. For the diuretic test, six treatment groups were administered 100, 200 and 400 mg/kg doses of rhizome and leaf extracts of the plant. The negative and positive control groups were treated with distilled water (2 mL/100 g) and furosemide (10 mg/kg), respectively. Cumulative urine volume, diuretic action, diuretic activity and saluretic index were then determined. In addition, virtual screening and molecular docking study of the compounds of the genus *Artemisia* were done.

Results: The rhizome and leaf extracts of *A. abyssinica* were found safe at a dose of 2000 mg/kg. Moreover, both extracts showed a significant diuretic action ($p < 0.05$). However, compared to the standard drug furosemide, the extracts had lower diuretic activity. The rhizome extract increased electrolyte excretion at all doses; particularly at the 200 and 400 mg/kg doses, it exhibited a profound natriuretic, chloruretic and kaliuretic effect with the concentration of 109 and 110 mmol/L for Na^+ , 93 and 106 mmol/L for Cl^- and 79 and 86 mmol/L for K^+ , respectively. These suggested inhibition of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ cotransporter as the potential mechanism of action of the extracts. Accordingly, virtual screening and a molecular docking analysis of the compounds of the genus *Artemisia* revealed that a few of them displayed a strong binding interaction with the cation-chloride cotransporter NKCC1 (PDB: 7S1Y), further indicating the cation-chloride cotransporter as a diuretic target of the constituents of the plant.

Conclusion: The current study supports the traditional claim of the plant for diuresis and recommends further isolation of the active constituents.

Key words: *Artemisia abyssinica*; Diuretics; *In silico*; *In vivo*; Furosemide.

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Introduction

Currently, cardiovascular diseases are the leading global cause of death. In 2021, it was estimated that hypertension affects 1.39 billion adult individuals,¹ while heart failure affects 64 million people worldwide.² These diseases are approximately equally prevalent in low and high income nations, 28.5 % and 31.5 %, respectively.³ According to Kibret and Mesfin,⁴ the prevalence of hypertension in Ethiopia is 19.6 %.

Diuretics are substances that increase urine and solute production by the kidneys. Most diuretics exert their action by decreasing renal tubular sodium reabsorption, thereby reducing the luminal-cellular osmotic gradient, which limits water reabsorption and results in diuresis.⁵ Different types of diuretics are clinically used for the treatment of various cardiovascular and renal disorders, such as hypertension, heart failure, ascites, acute kidney injury, ect.⁶⁻⁸ However, there are certain adverse effects to using a diuretic regularly. They cause electrolyte imbalances and metabolic abnormalities, including hyponatraemia, hypokalaemia or hyperkalaemia, hypomagnesemia, acid-base abnormalities, hyperuricaemia, hyperglycaemia, hyperlipidaemia and impotence.⁹ They also show a variety of drug-drug interactions with other medications.¹⁰

In addition to their adverse effects and drug-drug interactions, the affordability and availability of diuretics are a major problem in developing countries as they are long-term prescription drugs taken on a regular basis. Consequently, the majority of communities in developing nations utilise traditional medicine as an alternative or a combination therapy with a clinically approved diuretic regimen.¹¹ Therefore, scientific validation and standardisation of these traditional medical practices are important. *Artemisia* species are among the most commonly utilised traditional diuretic therapeutic plants in this regard. However, only a few of them have been studied pharmacologically for their diuretic properties.

Hence, the aim of this study was to analyse the *in silico* and *in vivo* diuretic activity of the 80 % methanol extracts of the rhizomes and leaves of *Artemisia abyssinica*, an indigenous Ethiopian medicinal plant that has a traditional diuretic claim.

Methods

Drugs

Drug and chemicals used in this study were: distilled water, normal saline (0.9 %), absolute methanol (*Carlo Erba*, Spain) and the standard drug furosemide (*Epharm*, Addis Ababa, Ethiopia).

Plant material

A. abyssinica was collected from the Wondo Genet Agricultural Research Centre found at Wondo Genet SNNRP, Ethiopia. The plant was authenticated by Mr Melaku Wondafrash, a senior botanist at the National Herbarium, College of Natural and Computational Sciences, AAU, where a voucher specimen was deposited (NA001 - *Artemisia abyssinica* Sch. Bip. ex A. Rich) for future references.

Experimental animals

Five to six-week-old healthy Swiss albino mice of either sex weighing 18–29 g were employed during the experiment. The mice were obtained from the animal house of the School of Pharmacy, Addis Ababa University and Ethiopian Public Health Institute and were kept at the School of Pharmacy animal house with a 12 h/12 h light/dark cycle. They were allowed access to water and pellets *ad libitum*. The handling of the mice and all procedures followed were in accordance with the Guide for the Care and Use of Laboratory Animals¹² and were approved by the Institutional Review Board of the School of Pharmacy, College of Health Sciences, Addis Ababa University.

Extraction

The dried powdered rhizomes (55 g) and leaves (200 g) of *A. abyssinica* were separately cold macerated with 80 % methanol for 72 h. The extracts were then filtered and the methanol was removed by a rotatory evaporator (*BUCHI Rota vapor R-200*, Switzerland). The remaining water portions of the extracts were freeze-dried using a lyophiliser (OPR-FDU-5012, Korea). Finally, the resulting dried 80 % methanol extracts were weighed, labelled and filled into sample vials and their percentage yield was calculated.

Acute toxicity

Acute oral toxicity tests of the 80 % methanol extracts were carried out as per the producers of the Organisation for Economic Co-operation and Development (OECD) guideline 425 (OECD,

2001). Ten female Swiss albino mice, five for each extract, were used for the test. First, a single mouse from each group was administered 2000 mg/kg of the extracts orally. The mice were fasted for 4 h before and 2 h after the extract administration. After dose administration, the mice were observed continuously for the first 4 h with 30 min intervals and until 24 h for any behavioural changes or signs of toxicity. Since no death was observed within 24 h, an additional four mice were successively administered the same dose of the extracts for the next four days and a similar procedure was followed. Eventually, each mouse was followed for 14 consecutive days with an interval of 24 h for the general signs and symptoms of toxicity and mortality.¹³

Diuretic activity

Diuretic activity was determined following a protocol applied by Lahlou et al¹⁴ with slight modifications. Eighty mice were randomly grouped into 10 groups: six treatment groups and two negative and two positive control groups, each with 8 mice. The mice were placed in a metabolic cage 24 h prior to the commencement of the experiment for acclimatisation and they were also fasted overnight with free access to water. The mice were then orally pretreated with normal saline (0.9 %) at a dose of 0.15 mL/10 g body weight to impose a uniform water and salt load.¹⁵ Then, each treatment group received 100 mg/kg, 200 mg/kg and 400 mg/kg of the 80 % methanol extracts of the leaves and rhizomes of *A abyssinica*. Negative controls were orally administered with the vehicles (distilled water) and positive controls were given furosemide (10 mg/kg) orally. After administration, urine output was measured every h for 5 h. Finally, the total urine collected was stored at -20 °C for further analysis.

The parameters: urinary excretion, diuretic action and diuretic activity were calculated by the formulas given below.

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

$$\text{Diuretic Action} = \frac{\text{Urinary excretion of treatment group}}{\text{Urinary excretion of control group}} \times 100 \%$$

$$\text{Diuretic Activity} = \frac{\text{Diuretic action of test drug}}{\text{Diuretic action of standard drug}} \times 100 \%$$

Analytical procedures

The electrolyte content (sodium, potassium and chloride) of the urine was determined using ion-selective electrode analysis (*COBAS 6000*, Roche, USA). Ratios of electrolytes; Na⁺/K⁺ and Cl⁻/K⁺Na⁺ were calculated to evaluate the saluretic activity of the extract. Moreover, the salt content of the extract had also been determined to rule out its contribution to urinary electrolyte concentration. In addition, pH was directly determined on fresh urine samples using a pH meter (*JENWAY 370*, England).

Statistical analysis

Data are presented as mean ± standard error of mean (SEM). The data were entered into Statistical Package for Social Sciences (SPSS) version 26.0 and one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare differences in mean among the groups. A p value of less than 0.05 was considered statistically significant.

Virtual screening and molecular docking study

Virtual screening and molecular docking study were carried out on crystal structures of Cation-chloride cotransporter NKCC1 (PDB: 7S1Y) using SeeSar13.0 software (*BioSolveIT*, Sankt Augustin, Germany). The ligands bumetanide found in the crystal structure of the 7S1Y was identified, the binding sites were selected for docking and its binding modes were calculated. The 206 compounds isolated from the genus *Artemisia* were loaded as sdf files and docking was carried out on the target protein in the docking mode. The best pose solutions were identified and the physiochemical parameters were computed. The HYDE score was used to estimate the binding affinity of the molecules.^{16, 17}

Results

Acute toxicity study

The acute oral toxicity test of the extracts of the leaves and rhizomes of *A abyssinica* did not show any signs of toxicity such as tremor, loss of weight, lethargy, paralysis, stress or other adverse behaviours or mortality within 14 days of follow-up. This entails that the LD₅₀ value of the extracts was above 2000 mg/kg in mice.

Diuretic activity

As shown in Table 1, the 80 % methanol rhizome extracts of *A. abyssinica* were found to have a significant diuretic action at all dose levels tested ($p < 0.05$). Particularly, following 5 h of therapy, the extracts had a significantly higher diuretic activity at 200 mg/kg and 400 mg/kg doses, 2.06 and 2.05, respectively, in comparison to the 100 mg/kg dose, 1.80. However, compared to the positive standard furosemide, the treatment

groups exhibited reduced diuretic action and activity. Similarly, the 80 % methanol leaf extracts of *A. abyssinica* showed significant diuretic action with values of 1.5 and 1.6 at 200 mg/kg and 400 mg/kg doses, respectively, after 5 h of treatment (Table 2). Nevertheless, the extract displayed no effect at 100 mg/kg and all the treated groups had lower activity than the positive control, the furosemide-treated group.

Table 1: Effect of 80 % methanol extracts of the rhizomes of *Artemisia abyssinica* on urinary volume excretion in mice

Group	Dose (mg/kg)	Urine volume (mL)					Diuretic action	Diuretic activity
		1 h	2 h	3 h	4 h	5 h		
Distilled water	0.2 mL	0.12 ± 0.04	0.37 ± 0.05	0.12 ± 0.03	0.00 ± 0.00	0.050 ± 0.02	1.00	-
AAR100	100	0.25 ± 0.06	0.00 ± 0.00	0.12 ± 0.02	0.00 ± 0.00	0.090 ± 0.03 ^{a, b, e*}	1.80	0.27
AAR200	200	0.50 ± 0.17 ^{a**b*e*}	0.43 ± 0.12	0.00 ± 0.00	0.30 ± 0.11 ^{a, b**e*}	0.103 ± 0.04 ^{a, e*}	2.06	0.80
AAR400	400	0.40 ± 0.12 ^{a*b*e*}	0.37 ± 0.08	0.30 ± 0.08 ^{a*b*c*}	0.20 ± 0.05 ^{a, b*}	0.102 ± 0.02 ^{a, b, e*}	2.05	0.82
Furosemide	10	0.75 ± 0.17 ^{a*, b**c*, d*}	0.40 ± 0.12	0.25 ± 0.08 ^{a*b*c**}	0.15 ± 0.04	0.120 ± 0.04 ^{a-d*}	2.40	1.00

Values are presented as mean ± SEM; n = 8; a = compared to negative control (Distilled water), b = compared to AAR100, c = compared AAR200, d = compared to AAR400, e = compared to furosemide; * ($p < 0.05$); ** ($p < 0.01$); AAR = 80 % methanol extract of the rhizomes of *Artemisia abyssinica*; numbers refer to doses in mg/kg/day.

Table 2: Effect of 80 % methanol extracts of the leaves of *Artemisia abyssinica* on urinary volume excretion in mice

Group	Dose (mg/kg)	Urine volume (mL)					Diuretic action	Diuretic activity
		1 h	2 h	3 h	4 h	5 h		
Distilled water	0.2 mL	0.13 ± 0.03	0.40 ± 0.03	0.12 ± 0.05	0.00 ± 0.00	0.05 ± 0.03	1.0	-
AAL100	100	0.13 ± 0.06	0.00 ± 0.00	0.07 ± 0.03	0.00 ± 0.00	0.05 ± 0.02	1.0	0.14
AAL200	200	0.45 ± 0.11 ^{a, b**e*}	0.40 ± 0.07 ^{e*}	0.00 ± 0.00	0.25 ± 0.05 ^{a, b, d, e*}	0.75 ± 0.05 ^{a, b, d, e*}	1.5	1.01
AAL400	400	0.50 ± 0.10 ^{a, b**e*}	0.45 ± 0.03	0.17 ± 0.07	0.13 ± 0.04 ^{a, b, c*}	0.08 ± 0.04 ^{a, b, c**e*}	1.6	0.72
Furosemide	10	0.72 ± 0.17 ^{a, b, c**d*}	0.51 ± 0.10 ^{a, b, c, *}	0.31 ± 0.09	0.16 ± 0.04 ^{a, b, c*}	0.13 ± 0.04 ^{a, b, d**c*}	2.5	1.00

Values are presented as mean ± SEM; n = 8; a = compared to negative control (Distilled water), b = compared to AAL100, c = compared AAL200, d = compared to AAL400, e = compared to Furosemide; * ($p < 0.05$); ** ($p < 0.01$); AAL = 80 % methanol extract of the leaves of *Artemisia abyssinica*; numbers refer to doses in mg/kg/day.

Saluretic activity

Urine electrolyte was measured from the pooled urine sample at the end of the fifth hour and the saluretic index was measured for all test samples. Accordingly, the rhizome extracts significantly increased electrolyte (Na^+ , Cl^- and K^+) excretion in a dose-dependent manner at all doses ($p < 0.05$) (Table 3). It showed a strong natriuretic and chloruretic effect at 200 and 400 mg/kg doses with concentrations of 109 and 110 mmol/L for Na^+ and 93 and 106 mmol/L for Cl^- , respectively. It also exhibited noticeably greater kaliuresis

with concentrations of 79 (200 mg/kg) and 86 mmol/L (400 mg/kg). However, compared to furosemide-treated groups, it has less electrolyte excretion. Furthermore, leaf extracts displayed the unexpectedly highest electrolyte loss at 100 mg/kg dose with natriuretic, chloruretic and kaliuretic concentrations of 120, 154 and 104 mmol/L, respectively (Table 4). Yet, at higher doses, it showed only mild natriuretic and chloruretic action (63 and 66 mmol/L) at 400 mg/kg.

Table 3: Effect of 80 % methanol extracts of the rhizomes of *Artemisia abyssinica* on 5 h urinary electrolyte excretion in mice

Group	Dose (mg/kg)	Urinary electrolyte concentration (mmol/L)			Saluretic Index*			Na ⁺ /K ⁺	Cl ⁻ /Na ⁺ + K ⁺
		Na ⁺	K ⁺	Cl ⁻	Na ⁺	K ⁺	Cl ⁻		
Distilled water	0.2 mL	64.0 ± 6.32	44.9 ± 0.35	74.4 ± 4.58	-	-	-	1.00	0.68
AAR100	100	97.0 ± 3.94 ^{a, e**d*}	67.1 ± 7.51 ^{a, c, d*te**}	95.0 ± 5.37 ^{d*ta, e**}	1.52	1.49	1.28	1.80	0.58
AAR200	200	109.0 ± 3.83 ^{a, e**}	79.5 ± 3.48 ^{b, c* a, e**}	93.5 ± 4.29 ^{a, c*te**}	1.70	1.77	1.26	2.06	0.50
AAR400	400	110.7 ± 6.27 ^{a, e**b*}	86.0 ± 5.92 ^{a**b, c, e*}	106.7 ± 3.86 ^{a**b, c, e*}	1.73	1.92	1.43	2.05	0.54
Furosemide	10	136.1 ± 6.74 ^{a, b, c, d**}	104.4 ± 5.98 ^{a, b**c, d**}	118.7 ± 4.65 ^{a, b**c, d*}	2.13	2.32	1.60	2.40	0.49

Values are presented as mean ± SEM; n = 8; a = compared to negative control (Distilled water), b = compared to AAR100, c = compared AAR200, d = compared to AAR400, e = compared to Furosemide; * (p < 0.05); ** (p < 0.01); AAR = 80 % methanol extract of the rhizome of *Artemisia abyssinica*; numbers refer to doses in mg/kg/day.

Table 4: Effect of 80 % methanol extract of the leaves of *Artemisia abyssinica* on 5 h urinary electrolyte excretion in mice

Group	Dose (mg/kg)	Urinary electrolyte concentration (mmol/L)			Saluretic Index*			Na ⁺ /K ⁺	Cl ⁻ /Na ⁺ + K ⁺
		Na ⁺	K ⁺	Cl ⁻	Na ⁺	K ⁺	Cl ⁻		
Distilled water	0.2 mL	48.0 ± 4.61	46.6 ± 6.35	54.2 ± 3.85	-	-	-	1.02	0.57
AAL100	100	120.0 ± 3.94 ^{a, c, d, **e*}	104.2 ± 7.51 ^{a, c, d, e**}	154.9 ± 5.37 ^{a, c, d**e*}	2.50	2.24	2.86	1.15	0.69
AAL200	200	43.0 ± 5.72 ^{b**d, e*}	35.0 ± 4.12 ^{b**d*}	45.3 ± 4.29 ^{b, e**d*}	0.89	0.75	0.84	1.23	0.58
AAL400	400	63.7 ± 2.83 ^{a, e**b**}	48.0 ± 4.52 ^{b**e**}	66.7 ± 3.86 ^{a, c*tb, e**}	1.33	1.03	1.23	1.33	0.60
Furosemide	10	88.0 ± 6.74 ^{a, c**b d*}	27.4 ± 5.98 ^{a, c, d*tb**}	98.0 ± 4.65 ^{a, c, d, **b*}	1.83	0.59	1.81	3.23	0.85

Values are presented as mean ± SEM; n = 8; a = compared to negative control (Distilled water), b = compared to AAL100, c = compared AAL200, d = compared to AAL400, e = compared to Furosemide; * (p < 0.05); ** (p < 0.01); AAL = 80 % methanol extract of the leaves of *Artemisia abyssinica*; numbers refer to doses in mg/kg/day.

Urinary pH

The urinary PH was measured from the pooled sample urine. Figure 1 indicated that there was no significant urinary PH disparity among all treatment and negative and positive control

groups after 5 h of treatment administration, except for the leaf extract-treated group at 200 mg/kg. It has significantly higher pH compared to just the untreated (negative control) groups.

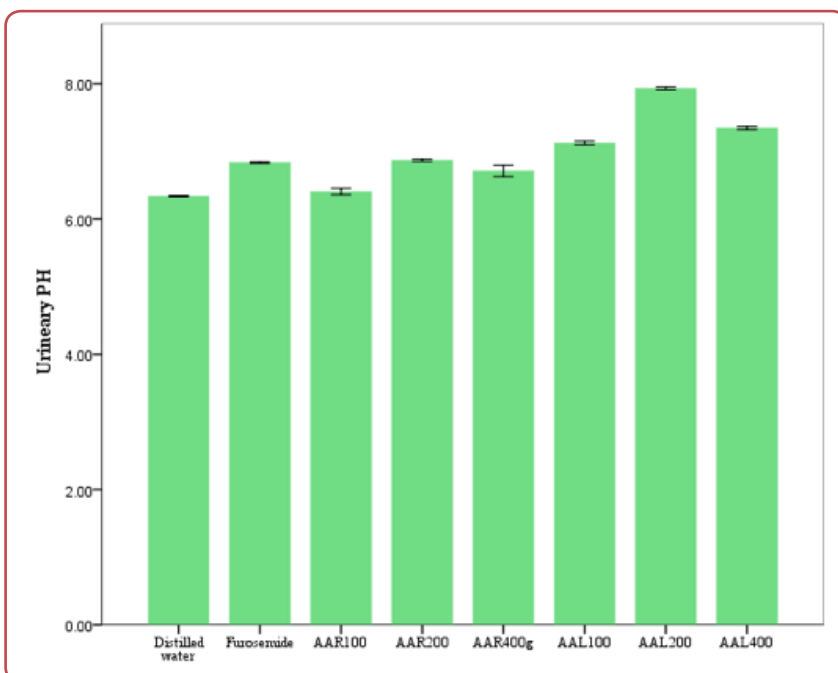


Figure 1: Urinary pH of mice treated with the 80 % methanol extracts of the rhizomes and leaves of *Artemisia abyssinica*

Values are presented as mean ± SEM; n = 8; AAR and AAL refer to 80 % methanol extracts of the rhizomes and leaves of *A abyssinica*, respectively; numbers refer to doses in mg/kg/day.

Virtual screening and molecular docking study

Virtual screening of 206 compounds that were previously reported from the genus *Artemisia* and a loop diuretic bumetanide was carried out on the crystal structures of the target protein Na⁺-K⁺-2Cl⁻ cotransporter (NKCC1) (cation-chloride cotransporter NKCC1) (PDB: 7S1Y). A few of the compounds showed strong binding interactions with the target protein. Interestingly, compounds 151 and 53 exhibited stronger affinity than even the ligand bumetanide. Moreover, they displayed

key hydrogen bond interactions with amino acid residues in the bumetanide binding site with HYDE scores of -42.5 and -40.4 kJ/mol. The binding modes of compounds to NKCC1 are shown in Figure 2. The Table 5 shows docking results and *in silico* physicochemical predictions of the four top-scoring compounds. All the compounds had optimum drug-like properties, such as Log P, in the range of 1 to 5.

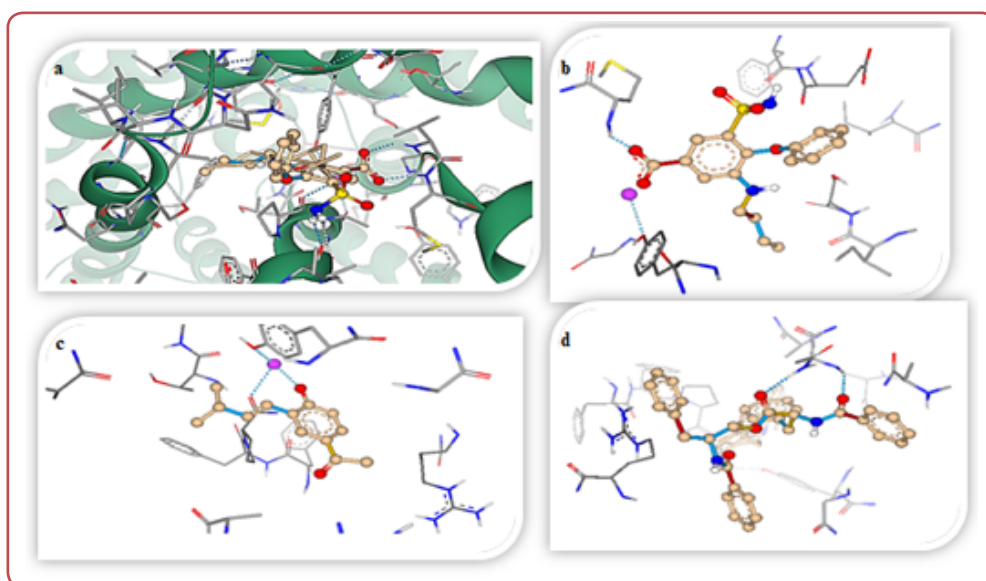


Figure 2: The binding modes of compounds to the cation-chloride cotransporter NKCC1 (PDB: 7S1Y) bumetanide binding site

a) Ribbon diagram of superimposed top-score compounds in the bumetanide binding site of NKCC1. Bumetanide, the template compound, is shown in the ball-stick model. b) Binding interaction of bumetanide with amino acid residues of NKCC1 c) Binding interaction of compound 53 with amino acid residues of NKCC1 d) Binding interaction of compound 197 with amino acid residues of NKCC1. Molecular docking was carried out using SeeSar13.0 software (BioSolveIT, Sankt Augustin, Germany).

Table 5: Docking results and prediction of partition coefficient Log P, aqueous solubility Log S and hERG toxicity of the top score compounds carried out using SeeSar13.0 software

Compounds	Structure	Δ GHYDE (kJ/mol)	Log P	Log S	Log D	hERG pIC ₅₀
Compound 151		-42.5	3.320	2.630	1.534	4.9000
Compound 53		-40.4	2.955	3.272	2.955	5.0670
Bumetanide		-39.6	2.522	2.357	-0.104	4.4921
Compound 197		-37.8	4.703	-0.224	4.703	4.8640

Discussion

The 80 % methanol rhizomes and leaf extracts of *A abyssinica* displayed moderate diuretic activity (0.72-1.00), especially at 200 and 400 mg/kg.^{18, 19} Similar urine output and electrolyte excretion results have been shown in previous *in vivo* diuretic studies on other *Artemisia* (L) species, such as *A annua*²⁰ and *A thuscula*.²¹ Moreover, the rhizome extract exhibited a higher dose-dependent diuretic action than the leaf extract, indicating a relatively abundant accumulation of active secondary metabolites in the rhizomes of the plant. In addition, the increased efflux of the electrolytes, especially the sodium, in the extract-treated groups *vis-à-vis* the negative control indicated the inhibition of the Na⁺-K⁺-2Cl⁻ symporter in the thick ascending loop of Henle within the kidney as the potential target for the diuretic mechanism of action of the constituents of the plant.^{22, 23} The less alkalinity of the urine sample and the higher Cl⁻/Na⁺K⁺ ratio further strengthen the suggestion of Na⁺-K⁺-2Cl⁻ (NK2CC) cotransporter as a diuretic target of the extracts than carbonic anhydrase, since carbonic anhydrase inhibition requires alkalinity of the urine and a lower Cl⁻/Na⁺K⁺ ratio. The *in silico* screening and molecular docking results also provided an additional insight into the diuretic mechanism of action of the extracts. To the best of authors' knowledge, this is the first study to examine the diuretic effects of 80 % methanol extracts of *A abyssinica*.

Conclusion

In conclusion, the rhizomes and leaves of *A abyssinica* proved to have diuretic activity. These, together with the absence of toxicity from the acute toxicity result, support their ethnobotanical claims. In addition, the virtual screening and molecular docking study of the compounds signify inhibition of Na⁺-K⁺-2Cl⁻ (NK2CC) cotransporter as possible mechanism of action of the diuretic constituents of the plant.

Ethics

This experiment was approved by the Institutional Review Board of the School of Pharmacy, College of Health Sciences, Addis Ababa University, decision No ERB/SOP/361/13/2021, dated 27 December 2021. The handling of the mice and all procedures followed were in accordance with the Guide for the Care and Use of Laboratory Animals.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request. The structure of compounds (1-206) isolated from the species of the genus *Artemisia* is available in the supplementary figure (Figure S1).

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References

- Jeemon P, Séverin T, Amodeo C, Balabanova D, Campbell NR, Gaita, D, et al. World heart federation roadmap for hypertension—A 2021 update. *Glob Heart.* 2021;16:1-31. doi: 10.5334/gh.1066.
- Savarese G, Becher PM, Lund LH, Seferovic P, Rosano GM, Coats AJ. Global burden of heart failure: a comprehensive and updated review of epidemiology. *Cardiovasc Res.* 2022;118:3272-87. doi: 10.1093/cvr/cvac013.
- Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. *Nat Rev Nephrol.* 2020;16:223-37. doi: 10.1038/s41581-019-0244-2.
- Kibret KT, Mesfin YM. Prevalence of hypertension in Ethiopia: A systematic meta-analysis. *Public Health Rev.* 2015;36:14. doi: 10.1186/s40985-015-0014-z.
- Wile D. Diuretics: A review. *Ann Clin Biochem.* 2012;49:419-31. doi: 10.1258/acb.2011.011281.
- Moore KP, Florence W, Pere G, Mauro B, Andreas O, Francesco S, et al. The management of ascites in cirrhosis: report on the consensus conference of the International Ascites Club. *Hepatology.* 2003;38:258-66. doi: 10.1053/jhep.2003.50315.
- Shah SU, Anjum S, Littler WA. Use of diuretics in cardiovascular disease: (2) Hypertension. *Postgrad Med J.* 2004;80:271-6. doi: 10.1136/pgmj.2003.010843.
- Felker GM, Ellison DH, Mullens W, Cox ZL, Testani JM. Diuretic therapy for patients with heart failure: JACC state-of-the-art review. *J Am Coll Cardiol.* 2020;75:1178-95. doi: 10.1016/j.jacc.2019.12.059.
- Sica DA. Current concepts of pharmacotherapy in hypertension: thiazide-type diuretics: ongoing considerations on mechanism of action. *J Clin Hypertens (Greenwich).* 2004 Nov;6(11):661-4. doi: 10.1111/j.1524-6175.2004.03902.x.
- Ferner R. Handbook of drug interactions: a clinical and forensic guide, 2nd edn. *Br J Clin Pharmacol.* 2012 Nov;74(5):893. doi: 10.1111/j.1365-2125.2012.04264.x.
- Misganaw M, Seboka N, Mulatu A. Documentation of traditional knowledge associated with medicinal animals in West Gojjam zone of Amhara region, Ethiopia. *Am J Life Sci* 2021;9:45-54. doi: 10.11648/j.ajls.20210903.12.
- National Research Council. Guide for the Care and Use of Laboratory Animals: Eighth Edition. Washington, DC: The National Academies Press, Washington, DC, 2011.
- OECD. Test no. 425: acute oral toxicity: up-and-down procedure, OECD guidelines for the testing of chemicals, Section 4. OECD Publishing, Paris, 2022.
- Lahlou S, Adil T, Zafar I, Badiia L. Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. *J Ethnopharmacol.* 2007;110:458-63. doi: 10.1016/j.jep.2006.10.005.
- Benjumea D, Abdala S, Hernandez-Luis, F, Pérez-Paz P, Martin-Herrera D. Diuretic activity of *Artemisia thuscula*, an endemic canary species. *J Ethnopharmacol* 2005a;100:205-9. doi: 10.1016/j.jep.2005.03.005.
- Schneider N, Hindle S, Lange G, Klein R, Albrecht J, Briem H, et al. Substantial improvements in large-scale redocking and screening using the novel HYDE scoring function. *J Comput Aided Mol Des.* 2012 Jun;26(6):701-23. doi: 10.1007/s10822-011-9531-0.
- Schneider N, Lange G, Hindle S, Klein R, Rarey M. A consistent description of HYdrogen bond and DEhydration energies in protein-ligand complexes: methods behind the HYDE scoring function. *J Comput Aided Mol Des.* 2013 Jan;27(1):15-29. doi: 10.1007/s10822-012-9626-2.
- Gujral ML, Saxena PN, Mishra SS. An experimental study of the comparative activity of indigenous diuretics. *JIMA.* 1955;25:49-51. PMID: 14392357.
- Vogel HG. Guidelines for the care and use of laboratory animals. in: drug discovery and evaluation: pharmacological assays 3rd edition. Berlin, Springer-Verlag; 2007. Pp. 2023-2029. doi: 10.1007/978-3-540-70995-4.
- Blanch LB, Spegazzini E. Diuretic Activity of *Artemisia annua* L. extracts, *Acta Far-m. Bonaerense.* 1998;17:131-4.
- Benjumea D, Abdala S, Hernandez-Luis F, Pérez-Paz P, Martin-Herrera D. Diuretic activity of *Artemisia thuscula*, an endemic canary species. *J Ethnopharmacol.* 2005b;100:205-9. doi: 10.1016/j.jep.2005.03.005.
- Bevevino LH, Aires MM. Effect of crude extract of roots of *Bredemeyera floribunda* Willd. II. Effect on glomerular filtration rate and renal tubular function of rats. *J Ethnopharmacol.* 1994 Jul 22;43(3):203-7. doi: 10.1016/0378-8741(94)90044-2.
- He D, He J, Zhou W. [Effect of furosemide on the carbonic anhydrase activity in vestibule]. *Lin Chuang Er Bi Yan Hou Ke Za Zhi.* 1998 Nov;12(11):510-2. Chinese. PMID: 11263227.