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EVALUATION OF DIURETIC ACTIVITY OF METHANOL LEAF EXTRACT OF CLERODENDRUM VOLUBILE IN SALINE-TREATED RATS

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ABSTRACT

*Clerodendrum volubil*e is used for the local management of fluid retention or edema. This present study aimed to evaluate the diuretic activity of the methanol leaf extract of *Clerodendrum volubil*e, (MCV) family - Labiatae on experimental animals at 200-800 mg/kg body weight respectively. The animals were observed for different indicators of diuresis such as volume, electrolyte concentration, and pH of urine for 5 hours using standard methods. Methanol leaf extract of *Clerodendrum volubile* (MCV) induced diuresis and produced a non-significant increase ($p<0.05$) in urine output when compared to the negative control group. The 200 mg/kg dose of MCV produced the highest volume. In addition, the extract produced non-significant increase in the saliuretic and natriuretic activity when compared with furosemide 10 mg/kg. MCV may still be considered to have favorable natriuretic effects since the computed values were above 2.0 which is the benchmark for natriuretic effects. From this study, it can be inferred that MCV may have diuretic activity thus justifying its ethnomedicinal use in the management of fluid over load.

KEYWORDS: ***Clerodendrum volubile*; Leaf; Diuretic; Saliuretic; Rats.**

INTRODUCTION

Medicinal plants have been discovered and used in traditional medicine since prehistoric times. Traditional healers in African ethnomedicines are well recognized for using a broad variety of plants in the treatment of parasitic diseases such as malaria, and a high proportion of herbal cures administered by traditional healers are widely regarded to be effective by their clientele. In some countries, the government promotes the use of traditional medicine rather than costly imported pharmaceuticals [1]. In developing countries, between 70 -95% of its population, depends solely on medicinal plants for treating and managing numerous disease states with the current trend worldwide now moving towards its use [2]. One of the application areas of herbal medicine is their use in diuresis. Several medicinal herbs have been identified to have significant diuretic effects [3] and this has been supported by some research papers published that evaluated the use of herbal medicines as diuretics [3]. Application of medicinal plants to produce diuresis are commonly employed to manage edematous conditions which includes heart failure, cirrhosis, nephrotic syndrome that can be attributed to body-fluid overload [4].

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The present-day orthodox drugs available as diuretics have numerous adverse effects. For instance, thiazide and loop diuretics lead to electrolyte abnormalities (hypokalemia, hyperuricemia and hyponatremia), acid base balance, metabolic abnormalities and acute hypovolemia [5]. Therefore, there is a current shift from orthodox medicine to alternative therapies especially from plant sources due to their safety, efficacy and cost effectiveness [2].

Clerodendrum volubile is a climbing and edible West African vegetable, belonging to the Labiatae family [6]. *Clerodendrum volubile* is predominantly cultivated for consumption wholly as a green leafy vegetable or as food condiment to improve soup taste, it is used for the local management of gouty arthritis, rheumatism, dropsy, swellings/edema, and ulcers [6,7]. The phytochemical contents of *Clerodendrum volubile* leaf extracts have been reported and was found to contain secondary metabolites such as alkaloids, flavonoids, saponins, anthraquinone, and cardiac glycosides [8].

The acute toxicity of *Clerodendrum volubile* was reported in literature and was not carried out in this study. The LD50 of methanol leaf extract of *Clerodendrum volubile* was reported to be greater than 3,000mg/kg [9].

Although *Clerodendrum volubile* is utilized in traditional African medicine as having numerous medicinal effects on various ailments, there are no scientific data regarding its diuretic effect to support the claimed ethnomedical use in fluid retention or edema. Therefore, this study aimed to provide scientific evidence for this claim and help in obtaining an alternative diuretic.

MATERIALS AND METHODS

Materials

Drugs, Assay kits and Chemicals

Furosemide (20 mg / 2 ml) Greenlife Pharma, Distilled water, Methanol, Assay kits for $Na^{+,}$ K^{+} and Cl^{-} Normal saline 0.9% (Drugfield).

Experimental Animals

Thirty (30) Wistar rats (120 g ±10 g) were used for this study. The animals were sourced from the Central Research Laboratory, University of Ilorin, and acclimatized in the animal house of the Department of Pharmacology and Toxicology, University of Ilorin, Kwara State, Nigeria. Animals were cared for according to the United States National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH publication No 85-23) and allowed free access to animal feed and water (ad-libitum). Ethical approval was gotten from the University of Ilorin Ethics Review Committee, and the experiment followed the Guidelines for laboratory Procedures laid down by the University of Ilorin Ethics Committee on Research, and also the International Animal Care and Use Committee (IACUC) in Nigeria. The Ethical approval number was FPS-ERC/ANS/2022/1.

Collection of plant materials

Clerodendrum volubile leaves were purchased in March 2021 from a local market in Ikale, in Okitipupa local government of Ondo state. Samples of the plant obtained were subjected to botanical identification and referencing at the University of Ilorin (UNILORIN) Herbarium, with the voucher specimen number UILH/01/019/1254 assigned. The leaves were air-dried under shade for two weeks and after which it was stored at room temperature.

Preparation of Methanol extract of *Clerodendrum volubile* leaf

The *Clerodendrum volubile* powdered material (200 g) was weighed into a clean jar, and extracted by maceration with 2.0 liters of methanol and distilled water in (70:30) for 72 h. The resultant extract was filtered and evaporated to dryness on a water bath at 45^{O}C and the concentrated extract stored in the refrigerator at -4^{O}C.

The filtrate was concentrated *in vacuo* at 45 ^{o}C using a rotary evaporator to give a residue of 28.21 g that corresponded to a yield percent of 14%. This was reconstituted in 0.9% (w/v) sodium chloride solution (physiological saline) to give the doses of 200, 400 and 800 mg/kg body weight of methanol extract of *Clerodendrum volubile* leaf (MCV) used in the present study.

Experimental Design

The procedure as previously described [10] with little modifications was adopted for this experiment. The bladder of the rats was emptied by gentle compression of the pelvic area and pulling of the tails before administration of plant extract and the drug. The animals were fasted from food but were provided with water for 18 h. All animals were treated with 0.9% normal saline intraperitoneally (i.p.) at 0 and 1 h before the study to ensure uniform water and salt load [11]. The animals were thereafter completely randomized into five groups of six animals each and various doses administered orally as follows:

Group A: Negative control (0.2 ml of Normal saline);
Group B: Positive control (10 mg/kg of Furosemide);
Group C: Extract (200 mg/kg of MCV);
Group D: Extract (400 mg/kg of MCV); and

Group E: Extract (800 mg/kg of MCV).

After the administration, each of the wistar rats were immediately placed in a metabolic cage kept at room temperature during the whole period of the experiment. In the course of the experiment, the animals were restricted to food and water.
The experimental procedures employed including the handling of animals was according to the Guidelines for the Care and Use of Laboratory Animals of the National Research Council (NRC 2011).

Collection of Urine and Determination of Diuretic Parameters

Urine was collected from the animals into graduated vials on an hourly basis for 5 h post-treatment. The volume of urine at each hour was noted and thereafter pooled together for each experimental group after the 5 h observation period. The urine samples were filtered to remove debris and shedding. Urine pH was determined on fresh urine samples collected using a calibrated pH meter [12,13].

Determination of Electrolyte Concentration in Urine

The concentration of Na^+ was determined by the colorimetry method using sodium monoliquid kit, the concentration of K^+ was be determined using colorimetric method using Potassium monoliqiuid kit. The concentration of Cl^- was determined using Chloride reagent kit, according to manufacturer's description. Urinary Parameters were computed statistically using the formula

Saliuretic activity = Na^++ Cl^-,

Natriuretic activity = Na^+/ K^+,

Natriuretic index = natriuretic activity in test group / natriuretic activity in negative control group.

Carbonic anhydrase inhibitory activity CAI = Cl^- / Na^+ + K^+,

Carbonic anhydrase inhibition index = CAI activity in test group/CAI activity in the negative control group.
Diuretic action or index = urine volume of test groups/urine volume of the control group.
Na^+index = Na^+ excretion in test group/ Na^+ excretion in the negative control group

K^+ index (Kaliuretic index) = K^+ excretion in test group/ K^+ excretion in the negative control group,
Cl^- index = Cl^- excretion in test group/ Cl^- excretion in the negative control group. Diuretic activity or Lipschitz value (diuretic action of extract/ diuretic action of reference drug) [14-16].

Statistical Analysis

The data were presented as the mean ± S.E.M. Results were analyzed statistically using GraphPad Prism (version 9.1.1). One-way analysis of one-way variance (ANOVA) was also used, after which Dunnett's test for parametric comparisons between the control and the treatment groups was used. The minimum level of significance was set at $P< 0.05$.

RESULTS

Demographic Characteristics and Risk Factor

Effect of methanol leaf extract of *Clerodendrum volubile* on urine volume pH

The methanol leaf extract of *C. volubile* at doses 200-800 mg/kg produced non-significant increase ($p>0.05$) in urine output compared to the normal control. Although the Furosemide treated group produced significant ($p<0.05$) urine output. All treatment groups showed no significant effect on urine pH.

Effect of Methanol leaf extract of *Clerodendrum volubile* on electrolyte concentration

The methanol leaf extract of *C. volubile* at doses 200-800 mg/kg produced non-significant increase ($p<0.05$) in electrolyte concentration compared to the normal control.

Effect of Methanol leaf extract of *Clerodendrum volubile* on urinary parameters

The methanol leaf extract of *C. volubile* at doses 200-800 mg/kg produced natriuretic activity and saliuretic activity although non-significant increase ($p<0.05$) when compared to the normal control. The Furosemide treated group produced a significance ($p<0.05$) increase in saliuretic and natriuretic activity.

Effect of the methanol leaf extract of *Clerodendrum volubile* on urinary parameters

There was a significant difference ($p<0.05$) in the chloride index of the group administered with 400 mg/kg of MCV when compared to the normal control group. The methanol leaf extract of *C. volubile* at doses 200-800 mg/kg produced although non-significant increase ($p<0.05$) in sodium, potassium and carbonic anhydrase inhibitory index.

Effect of the Methanol leaf extract of *Clerodendrum volubile* on more urinary parameters

The diuretic index of MCV at doses 200-800 mg/kg were above the benchmark 1.50 required as a measure of the diuretic potential of a chemical compound.

Table 1: Effect of Methanol leaf extract of *Clerodendrum volubile* on urine volume pH

Treatment/Dose	Urine Volume (ml)	Urine pH
Normal saline	0.80 ± 0.15	6.43 ± 0.28
Furosemide (10 mg/kg)	2.42 ± 0.47*	6.35 ± 0.28
Extract (200 mg/kg)	1.26 ± 0.16$^{n.s}$	6.34± 0.24
Extract (400 mg/kg)	1.20 ± 0.25$^{n.s}$	6.26 ±0.18
Extract (800 mg/kg)	1.21 ± 0.30$^{n.s}$	6.14 ±0.09

n=6, values are expressed as Mean ± S.E.M.*p<0.05, compared with control;
n.s: non-significant

Table 2: Effect of Methanol leaf extract of *Clerodendrum volubile* on electrolyte concentration

Treatment/Dose	K+ ion (mmol/L)	Cl^- ion (mmol/L)	Na+ion (mmol/L)
Normal saline	14.05 ± 0.44	32.61 ± 3.05	80.32±3.68
Furosemide (10 mg/kg)	13.41 ± 0.50	41.22 ± 3.80	86.67±3.40
Extract (200 mg/kg)	13.87 ± 0.56	34.07 ± 2.22	88.79±3.87
Extract (400 mg/kg)	14.13 ± 0.53	32.80 ± 3.36	85.31±2.45
Extract (800 mg/kg)	14.58 ± 0.48	35.97± 2.51	85.45±2.78

n=6, values are expressed as Mean ± S.E.M.

Table 3: Effect of Methanol leaf extract of *Clerodendrum volubile* on urinary parameters

Treatment	Dose (Mg/kg)	Saliuretic (Na/Cl-)	Natriuretic (Na+/K+)	Saliuretic Index	Natriuretic Index
Normal saline		112.9 ±2.48	5.77± 0.17	1.00	1.00
Furosemide	10	127.9±3.51*a	6.47±0.11*a	1.133±0.03	1.129±0.03
Extract	200	122.9±4.14	6.43±0.33	1.091±0.05	1.125±0.08
Extract	400	118.1±3.94	5.95±0.15	1.049±0.05	1.038±0.05
Extract	800	121.4±1.01	5.87±0.14	1.078±0.03	1.021±0.03

n=6, values are expressed as Mean ± S.E.M. *p<0.05, a-comparison of negative control with positive control.

Table 4: Effect of the Methanol leaf extract of *Clerodendrum volubile* on urinary parameters

Treatment	Dosage Mg/kg	Na+ index	Cl- index	K+ index	CAI index
Normal saline		1.00	1.00	1.00	1.00
Furosemide	10	1.08±0.04	1.27±0.05	0.96±0.02	1.20±0.07
Extract	200	1.11±0.06	1.07±0.06	0.99±0.02	0.98±0.07
Extract	400	1.07±0.05	1.01±0.05*a	1.03±0.01	0.95±0.04
Extract	800	1.07±0.03	1.12±0.05	1.05±0.03	1.06±0.05

n=6, values are expressed as Mean ± S.E.M.*p<0.05; a-comparison of 400 mg/kg of extract with positive control. CAI: Carbonic anhydrase inhibitory activity.

Table 5: Effect of the Methanol leaf extract of *Clerodendrum volubile* on more urinary parameters

Groups	Diuretic Index/Action	CAI($Cl^-/Na^+ + K^+$)	Diuretic Activity
Normal saline	1.00	0.35 ± 0.04	1.00
Frusemide (10 mg/kg)	3.33 ± 0.72	0.42 ± 0.04	3.33 ± 0.72
Extract (200 mg/kg)	1.89 ± 0.45	0.34 ± 0.03	0.72 ± 0.21
Extract (400 mg/kg)	1.58 ± 0.26	0.33 ± 0.03	0.75 ± 0.34
Extract (800 mg/kg)	1.96 ± 0.62	0.37± 0.03	0.65 ± 0.19

n=6, values are expressed as Mean ± S.E.M. CAI: Carbonic anhydrase inhibitory activity.

DISCUSSION

Diuretics are designed to increase the amount of water and salt expelled from the body as urine. Consequently, by reducing the build-up of fluid, diuretics also lower blood pressure [5].

In the present study, methanol leaf extract of *Clerodendrum volubile* (MCV) was found to induce diuresis and increase urine output (non-significant) when compared to the negative control group. At a dose of 200 mg/kg, the extract produced more volume of urine suggesting that urine production by MCV was not in a dose-dependent manner. Although, the volume of urine produced by the treated animals was not significant when compared to the standard drug used furosemide (10 mg/kg). There was a significant ($p<0.05$) increase in urine volume produced by the animals treated with the standard drug when compared to the negative control group. Furosemide is a widely used diuretic in clinical practice as a standard drug to compare pharmacological response [17].

Urinary pH measurement revealed that the different treatment groups that received test doses of MCV including the negative and positive control groups produced slightly acidic urine [18]. The average urine pH for the Wistar rats ranged from 6.0 - 6.4 values. However, there was no statistically significant change in urinary pH [18]. This finding with respect to urine pH in this study was similar to those previously reported [5].

The urine samples collected over the five hours were analyzed for electrolyte content (Na^+, K^+, and Cl^-). Urinary Na^+ excretion of animals treated with MCV at doses 200 - 800 mg were increased when compared with the negative control group [19], but were not significant. In addition, there was also a non-significant increase in Cl for all the extract treated groups. However, there was a significant increase in animals treated with furosemide ($p<0.05$).

Saliuretic activity is an indicator of the amount of Na^+ and Cl^-, whereas the ratio of Na^+ and K^+ indicates natriuretic activity. The Na^+/K^+ ratio is a translatable biomarker of mineralocorticoid receptor antagonist but also a good indicator of natriuretic activity [20]. Furthermore, a Na^+/K^+ ratio greater than 2 shows the ability of a chemical compound to excrete a greater proportion of sodium ions in contrast to potassium ions [21]. Results obtained from the present study showed that there was no significant difference ($p<0.05$) between the saliuretic activity of the treatment groups and the control group suggesting that the concentration of Na^+ and Cl^- ions in the urine samples analyzed were of minimal quantity. In addition, increased natriuretic activity observed with MCV (200 mg/kg) suggests an excretion of more sodium ions than potassium ions. The calculated saliuretic ($Na^+ + Cl^-$) and natriuretic activities gave a value of 2.0 indicating that MCV might possess favorable natriuretic effects [16]. The diuretic activity attributed to MCV in this present study may be due to the increased natriuretic and saliuretic activities as well as reduced carbonic anhydrase inhibitory activities similar to what was previously documented [5].

The diuretic index, which is an indicator of the diuretic potential of a chemical compound, is considered to be good if the values are greater than 1.50, moderate if between 1.00 and 1.50, mild when it lies between 0.72 and 1.00, and absent if less than 0.72 [14]. Therefore, the computed diuretic index of MCV was higher than the 1.50 benchmark thus it can be inferred, that MCV may have diuretic activity. The computed kaliuretic index, Na^+ index and Cl^- index for MCV were higher than the physiological saline-treated animals, consistently emphasize the good potential diuretic activity of the *C. volubile* leaves.

The findings may suggest that diuretic activity of MCV could be mediated by inhibiting tubular reabsorption of water and anions, as it has been suggested for some plants such as *Spergularia purpurea* and the roots of *Carica papaya* and *A. comosus* [22,23]. Previous report had shown that the aqueous and alcoholic extract of *C. volubile* leaf contained glycosides, tannins, phenolic compounds, resins, alkaloids, and proteins [24]. The primary active metabolites from medicinal plants that have been reported to produce diuretic activity are essential oils, flavonoids and saponins [25].

CONCLUSION

The data obtained from the present study suggest that methanol extract of *C. volubile* leaf produced diuretic activity in female Wistar albino rats and thus supports the age-long claim of the use of the plant as diuretic agents. The presence of secondary metabolites like flavonoids which facilitate the release of renal prostaglandin might be responsible for the diuretic action of the plant. The plant may be explored in the development of complementary and alternative diuretics.

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