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# Diuretic and antioxidant activities of aqueous stems and leaves extracts of *Sida veronicaefolia* (lam), *Nelsonia canescens* (lam) *spreng* and *Crassocephalum bauchiense (*hutch.) Milne–redh on female wistar rats

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**Abstract**
The objective was to study the diuretic and antioxidant activities of aqueous extract of stems and leaves of *Sida veronicaefolia* (AESLSV), *Nelsonia canescens* (ASLENC) and *Crassocephalum bauchiense* (AESLCB) 14 groups of female rats were used to study the diuretic activity of extracts. Groups I and II serves as controls (and other Groups received different doses of plants extracts. y after the treatment, all the rats were placed in to the metabolic cages for rat urines collection. Finally, different urinary parameters were measured. The antioxidant activities were carried out by the DPPH and FRAP tests.
The AESLSV, AESLNC and AESLCB at different doses have significantly increased the percentage of urinary excretion and urinary excretion levels of electrolytes. A dose dependent decrease of the aldosterone index was observed in animals treated with different doses of AESLNC and AESLCB. Since other index evaluated did not change, the AESLSV would act as a loop diuretic while the two other extracts would act as thiazides.
At the end of the DPPH reduction test, he $IC_{50}$ of different extracts were 204.17; 29.36 and 35.30 µg/ml respectively for AESLSV, AESLNC and AESLCB. The FRAP of ours extracts was low compared to those vitamin C. This means that AESLSV have low *in vitro* antioxidant potential while AESLNC and AESLCB have moderate potential.
The results obtained show that the different plant extracts possess diuretic and anti-oxydant activities. Consequently, their use as diuretic is justified.

**Keywords:** Pregnancy, water and salt retention, *Sida veronicifolia*, *Nelsonia canescens*, *Crassocephalum bauchiense,* diuretics, antioxydants

## Introduction
Pregnancy causes many anatomical, physiological, and metabolic and biochemical changes in the mother [1, 2]. In the majority of cases, these changes are associated with benign discomfort (nausea, vomiting, edema, fatigue.). But, in some cases, these discomforts can induce serious complications that could have severe consequences on the health of the mother and/or the fœtus she carries and eventually lead to their death.
This is the case for hemodynamic changes that lead to an increase in plasma volume due mainly to sodium and water retention in the kidneys. These variations sometimes lead to the formation of oedemas (especially of the lower limbs) and the development of gestational hypertension with the help of oxidative stress generated by the placenta [3, 4]. This further exposes the mother to the subsequent development of cardiovascular disease. Indeed, studies have proven that a woman who has developed cardiovascular disease during pregnancy has a high chance of developing it later [5].
Thus, every year about 10 million women suffer from injuries, infections or diseases related to pregnancy or childbirth and more than one hundred thousand women and four million newborns under one month old die as a result of pregnancy and/or childbirth complications [6, 7, 8].
Given the side effects and teratogenic effects generated by synthetic drugs on pregnancy, their use during pregnancy is drastically limited, favoring a return to herbal medicine [9].
Thus, in search of alternative treatments for their ailments, pregnant women use medicinal plants which in the majority are still unknown to the public and have not been subject to any pharmacological studies [10, 11]. Ours previous work was aims to carry out an inventory of medicinal plants used for the treatment of child-bearing ailments in some localities of the Menoua Division (West Region of Cameroon).

It permit ours to identify, 88 medicinal plants used in 122 recipes for the treatment of 24 ailments were recorded [12]. *Sida veronicifolia*, *Nelsonia canescens* and *Crassocephalum bauchiense* were among the most used for the treatment of excessive fluid and salt retention which was the most mentioned pregnancy-associated discomfort.

Drugs that increase the rate of urine flow and sodium excretion are called diuretics. These drugs are used to adjust the volume and composition of body fluids in a variety of clinical situations. In fact, drug-induced dieresis is beneficial in many life threatening disease conditions such as pregnancy toxaemia, hypertension, renal failure, congestive heart failure, nephritic syndrome, cirrhosis [13].

That is why the present study aims to evaluate the pharmacological properties of three of the most commonly used plants for the treatment of excessive water and sodium retention.

## Materials and Methods

### Plants collection and authentification

The fresh plants of *Sida veronicaefolia* (Lam.) (Malvaceae), *Nelsonia canescens* (Lam) *Spreng* (Acanthaceae) and *Crassocephalum bauchiense* (Asteraceae) were collected at the vegetative stage in August 2014 in Bamendou village, Menoua Division, West Region of Cameroon. The taxonomic identification of the plants was authenticated by the Cameroon National Herbarium (CNH) where the voucher specimen (No. 29 010/SRF/Cam, 6 898/SRFK and 37 884/HNC respectively) are deposited.

### Preparation of plant extracts

The stems and leaves of each plant were collected, dried and powdered using a mechanical grinder. 100g of powder of each plant was macerated in 1l of distilled water for 24 hours at room's temperature. After, extracts were filtrated using N°1 whatmann paper and vaporized at 45°C using hot oven.

### Evaluation of Diuretic Activity

### Animals

All animals were procured and housed in animal house under standard animal house conditions and allowed free access to food and water. Animals used were female Wistar rats of 12-14 weeks old and weighing between 170 and 200 g.

### Animals Treatment

The method of Murugesan *et al*., 2000 [14] was used. According to this method, eighty-four (84) rats aged 12 to 14 weeks and weighing between 170 and 220 g, fasting from food for 18 hours were divided into 14 groups of 6 animals each and received a physiological solution of 0.9% NaCl at a dose of 25 ml/kg to impose a uniform water and salt balance to these animals. The animals were then given distilled water at a dose of 1 ml/100 g of body weight for group 1 (neutral control) or furosemide at a dose of 20 mg/kg for group 2 (positive control) or different doses of the different extracts (treated groups). These last groups of animals received: AESLSV at the doses of 30 mg/kg (Group 3); 60 mg/kg (Group 4); 120 mg/kg (Group 5); 240 mg/kg (Group 6) or AESLNC at the doses of 41.25 mg/kg (Group 7); 82.5 mg/kg (Group 8); 165 mg/kg (Group 9); 330 mg/kg) (Group 10) or AESLCB at 30 mg/kg (Group 11); 60 mg/kg (Group 12); 120 mg/kg (Group 13) and 240 mg/kg (Group 14). After treatment, each rat was placed alone in a metabolic cage where its urine was collected and its excreted urine volume measured after 6 hours. Finally, the collected urine was used to determine the following urinary parameters: total urinary volume excreted, conductivity, pH, electrolyte concentrations ($Na^+$, $K^+$, $Cl^-$, $HCO_3^-$).

The concentrations of $Na^+$ and $K^+$ were determined with flame photometry while those of $Cl^-$ and bicarbonate were estimated by titrimetry.

**The data obtained were used to determine**

- Percentage of urinary excretion (total volume of urine collected/total fluid administered x 100);
- Diuretic action (total urine volume of treated rats/total urine volume of neutral control rats)
- Diuretic activity (diuretic action of test extract/diuretic action of standard drug);
- Aldosterone secretion index ($Na^+/K^+$);
- -Thiazide diuretic index ($Na^+/Cl^-$);
- Carbonic anhydrase inhibition index [$Cl^-/(Na^+ + K^+)$];
- Anionic gap [$(Na^+ + K^+)-Cl^-$];
- Renal Electrolyte Excretion Fraction (REEF)

REEF= $(V_i x Ci)/(Vo \times Co)$ with
VI = Urine volume of group test i
Ci = Concentration of considered electrolyte of group test i
VO = Urine volume of neutral control
Co = Concentration of considered electrolyte of neutral control I = Volume dourine du test
Ci = Concentration en électrolyte considéré du test
VO = Volume durine du temoin négatif
Co = concentration en électrolyte considéré dutemoin négatif

### Evaluation of anti-oxidant activity

### Diphenyl-2 picrylhydrazyl (DPPH) reduction test

The DPPH (1, 1-diphenyl-2-pycrilhydrazyl) assay was performed according to the method described by El-Ghorab *et al*. (2006) [15]. For each plant extract, a stock solution (2 mg/ml) was first prepared and then underwent a series of dilutions with methanol. Subsequently, 100 µL of each diluted extract was pipetted, introduced into a test tube and 900 µL of a previously prepared methanolic solution of DPPH (0.3 mM) was added to it so as to obtain for each dilution series the following concentrations: 12.5; 25; 50; 100 and 200 µg/ml.

The tubes were incubated in the dark for 30 minutes at room temperature and the optical density was measured at 517 nm. Ascorbic acid (vitamin C) was used as a positive control. Three trials were performed for each extract and the results were expressed as percent radical scavenging calculated from the formula:

$$\text{Scavenging}\,(\%) = [(A_0 - A_1)/A_0]\,X100$$

With: $A_0$ = optical density of DPPH reagent
$A_1$ = optical density of the mixture (extract + DPPH)

The percentages of free radical scavenging were then expressed as a function of the logarithms of the corresponding concentrations of each sample. Thus, a linear regression line was plotted for each sample from these data and used to determine the IC50 which is the sample concentration required to reduce 50% of the total free DPPH radicals [16].

**FRAP (Ferric Reducing Antioxidant Power)**

**Table 1:** The assay was carried out as described in the following

| Reagents | Tubes | | | | |
|---|---|---|---|---|---|
| Final concentration of extract or reference compound (µg/ml) | 12,5 | 25 | 50 | 100 | 200 |
| Volume of extract (2000µg/ml) (µl) | 25 | 50 | 100 | 200 | 400 |
| Phosphate buffer (pH=6,6) (µl) | 475 | 450 | 400 | 300 | 100 |
| $[K_3Fe(CN)_6]$ 1% (µl) | 500 | 500 | 500 | 500 | 500 |
| **Incubate at 50 °C for 20 minutes** | | | | | |
| Trichloroacetic acid 10% (µl) | 500 | 500 | 500 | 500 | 500 |
| | Centrifuge at 3000 rpm for 10 minutes | | | | |
| Supernatant of corresponding tube (µl) | 500 | 500 | 500 | 500 | 500 |
| Distilled water (µl) | 500 | 500 | 500 | 500 | 500 |
| $FeCl_3$ 0.1% in methanol (µl) | 100 | 100 | 100 | 100 | 100 |
| **Read the optical density of each tube at 700nm** | | | | | |

The increase in absorbance of each extract with its concentration was considered to correspond to the iron reducing power of the latter

**Statistical Analysis**

Experimental results were expressed as mean ± SEM (n=6). The data were analyzed by SPSS software. These data were analyzed by the one way ANOVA (Analysis of Variance) test and the differences between the Means established by the Fisher SSD (Smallest Significant Difference) test.

**Results and Discussion**

**Results**

The AESLSV, AESLNC and AESLCB were subjected to quantitative phytochemical tests to identify and quantify the phytoconstituents, and it revealed the presence of phenolic compounds, and flavonoids in all extracts but, the AESLNC is the richest, followed by the AESLCB and that the AESLSV contains the least.

**Table 2:** Levels of flavonoïdes and total phenols of extracts

| Nom de la plante | Total phenols (mg EAG/g) | Flavonoïdes (mg EC/g) |
|---|---|---|
| *S. veronicifolia* | 1.27±0.15 | 0.47±0.02 |
| *N. canescens* | 4.34±0.15 | 0.52±0.01 |
| *C. bauchiense* | 2.68±0.28 | 0.49±0.01 |

Values are the mean ± SEM of 3 replicates; GAE, galic acid equivalent; EC, catechin equivalent.

**Diuretic Effects of different aqueous extracts**

**Effects of the different extracts on the percentage of urinary excretion**

Fig 1 shows the evolution of the percentage of urinary excretion of treated rats compared to the neutral control. It can be seen that the treatment resulted in an increase in the percentage of urinary excretion in all treated animals with significant increases at doses of: 30 ($P< 0.001$) and 60 mg/kg ($P< 0.01$) for AESLSV; 41.25 mg/kg ($P< 0.001$) for the AESLNC and 30, 240 ($P< 0.01$) and 60 mg/kg ($P< 0.001$) for AESLCB.

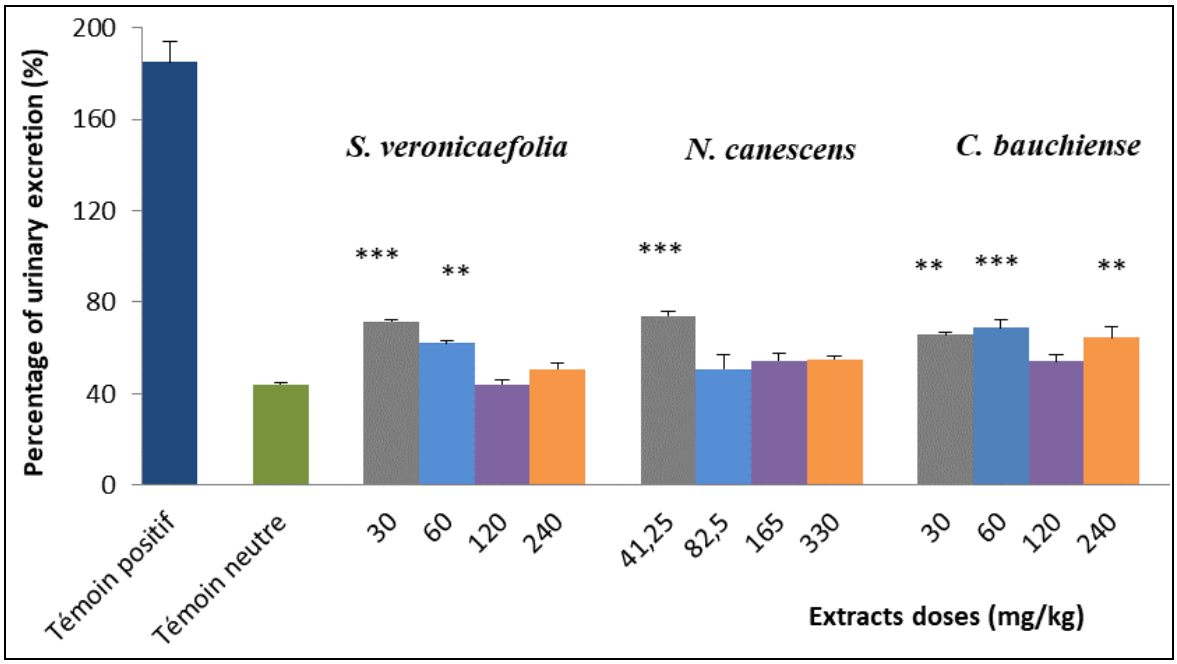


**Fig. 1:** Effect of treatment on the percentage of urinary excretion of control animals and those treated with different doses of AESLSV, AESLNC and AESLCB. Each value represents the Mean ± SEM of six animals per group; **; *** Values significantly different at the respective thresholds of $P< 0.01$; $P< 0.001$ from those of the neutral control group (Fisher SSD Test);

**Effects of the different extracts on urinary pH, urinary conductivity and urinary electrolyte levels**

Table 3 shows that compared to the neutral control, the administration of the different extracts resulted in a dose-independent increase in conductivity and in the quantity of urinary electrolytes in the treated animals.

Indeed, AESLSV induced a significant increase in urinary conductivity at 30 ($P< 0.01$), 60 ($P< 0.05$) and 120 mg/kg ($P< 0.01$). Also, a significant increase ($P< 0.01$ and $P< 0.001$) in sodium, chloride and bicarbonate levels was noted in all treated animals except those given AESLSV at 30 mg/kg. Potassium levels were significantly ($P< 0.05$) increased at both highest doses.

AESLNC induced a significant increase in urinary conductivity at 41.25 mg/kg ($P< 0.001$) and 330 mg/kg ($P< 0.05$) and in the levels of all electrolytes at different doses. A significant increase of potassium level was noted at the doses of 165 ($P< 0.05$) and 330 mg/kg ($P< 0.001$). Also a significant increase of sodium ($P< 0.001$; $P< 0.05$ and $P< 0.01$) and bicarbonate levels ($P< 0.001$) was observed at all doses except the last dose. For chloride level, it was significant ($P< 0.001$ and $P< 0.01$) at all doses except the dose of 41.25mg/kg.

For the AESLCB, treatment resulted in a significant increase in conductivity at 60 mg/kg ($P< 0.01$), 30 mg/kg and 240 mg/kg ($P< 0.05$) and in the levels of all electrolytes. Sodium levels were significantly increased at 60 mg/kg, 120 mg/kg ($P< 0.01$) and 240 mg/kg ($P< 0.001$). Potassium and chloride levels were significantly increased ($P< 0.05$; $P< 0.01$) only at 240 mg/kg and 60 mg/kg respectively. Bicarbonate levels were increased ($P< 0.001$) at all doses except 120 mg/kg.

**Effects of treatment on urine volume, diuretic action, diuretic activity, and REEF**

In general, there was an increase in all parameters evaluated in all treated animals, but this increase was not significant in all groups. Indeed, the increase in urine volume was significant at the doses: 30 ($P< 0, 01$) and 240 mg/kg ($P< 0, 05$) for AESLSV; 41.25 mg/kg ($P< 0.01$) for AESLNC and at 30 mg/kg ($P< 0.05$), 60, and 240 mg/kg ($P< 0.01$) for AESLCB (Table 4).

For REEFs, the increase was significant ($P< 0.05$; $P< 0.01$; $P< 0.01$) for all electrolytes and in all groups except the groups receiving AESLCB at doses of 120 and 240 mg/kg for chloride REEF and bicarbonate REEF respectively (Table 4).

**Effects of the different extracts on the diuretic indices and the anion gap**

Compared to the neutral control, a dose-independent decrease of the anion gap is observed with the AESLSV and AESLNC. For AESLSV, the decrease was significant ($P< 0, 01$ and $P< 0, 05$) at all doses except the lowest while for AESLNC, it was significant ($P< 0.001$) at 82.5 and 330 mg/kg.

Also a highly significant decrease ($P< 0.001$) in the aldosterone secretion index was noted at the highest dose of AESLNC and EASLCB. This decrease was also significant at the 120 mg/kg dose of AESLCB. Regarding the indices of thiazide secretion and carbonic anhydrase inhibition, no significant variation was observed on these parameters for all treated animals compared to the neutral control. As for the $HCO3^-/Cl^-$ ratio, it was significantly increased ($P< 0.01$) at 82.5 and 30 mg/kg of the AESLNC and AESLCB, respectively (Table 5).

**Table 3:** Effects of treatment on urine pH, urine conductivity and urine electrolyte levels

| Plants | Dose (mg/kg) | Urine pH | Urine Conductivity | Potassium level (mmol/l) | Sodium level (mmol/l) | Chloride level (mmol/l) | bicarbonate level (mmol/l) |
|---|---|---|---|---|---|---|---|
| Control | Neutral (0) | 6.32±0.09 | 5.18±0.56 | 19.63±0.89 | 124.83±2.26 | 368.00±21.66 | 44.00±2.24 |
| | Positive (20) | 7.14±0.3 | 18.79±.14*** | 27.95±2.15** | 157.23±1.67*** | 483.20±13.52** | 61.44±2.78*** |
| *S. Veronicaefolia* | 30 | 6.46±0.37 | 6.58±0.57 | 21.04±0.35 | 122.43±1.62 | 372.80±9.38 | 71.68±3.84*** |
| | 60 | 6.32±0.17 | 8.19±1.4* | 20.41±0.48 | 142.48±3.30*** | 466.67±21.50*** | 82.56±2.25*** |
| | 120 | 6.63±0.37 | 9.16±1.37** | 21.89±0.91* | 145.96±3.39*** | 450.00±6.32** | 68.80±1.01*** |
| | 240 | 6.62±0.31 | 8.95±1.17** | 21.66±0.37* | 148.17±3.43*** | 450.00±15.92** | 66.56±1.92*** |
| *N. canescens* | 41.25 | 6.66±0.14 | 10.39±0.52*** | 21.17±0.50 | 146.76±3.05*** | 370.33±6.32 | 70.23±1.44*** |
| | 82.5 | 6.73±0.33 | 6.08±1.30 | 23.82±3.75 | 133.07±2.36* | 477.14±26.22*** | 111.90±8.01*** |
| | 165 | 6.34±0.40 | 7.39±1.29 | 28.31±4.11***** | 136.12±3.60** | 456.00±16.65***** | 72.80±3.13*** |
| | 330 | 6.03±0.21 | 8.19±1.57* | 39.58±3.49******* | 132.31±2.28 | 600.00±14.60******* | 53.60±1.73 |
| *C. bauchiense* | 30 | 6.57±0.16 | 9.04±1.90* | 20.70±0.59 | 132.43±2.01 | 360.00±25.30 | 81.60±2.11*** |
| | 60 | 6.92±0.17 | 10.76±1.51** | 24.51±3.03 | 134.31±2.41** | 413.33±5.96* | 75.20±1.75*** |
| | 120 | 6.66±0.20 | 5.16±0.25 | 27.00±4.47* | 137.03±4.55** | 370.67±6.34 | 54.60±1.10 |
| | 240 | 7.33±0.29 | 8.43±1.07* | 32.50±5.01***** | 147.92±1.63*** | 380.00±7.30 | 93.87±12.11*** |

Values represent Means ± ESM of six animals per group; *; **; ***Values significantly different at respective thresholds of $P< 0.05$, $P< 0.01$, $P< 0.001$ from those of the control-neutral group (Fisher S.S.D. Test)

**Table 4:** Effects of treatment on urine volume, diuretic action, diuretic activity and REEF (Renal Electrolyte Excretion Fraction)

| Plants | Dose (mg/kg) | Urine Volume (ml) | Diuretic Action | Diuretic activity | REEF | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Potassium | Sodium | chlorure | bicarbonate |
| Control | Neutral | 2.77±0.09 | 1.00±00 | 0.25±00 | 1.00±00 | 1.00±00 | 1.00±00 | 1.00±00 |
| | Positive | 10.90±1.37*** | 3.93±0.12 | 1.00±00 | 3.52±0.08*** | 3.93±0.21*** | 3.50±0.13*** | 3.61±0.28*** |
| *S. Veronicaefolia* | 30 | 5.75±0.50** | 2.07±0.11 | 0.53±0.03 | 2.18±0.13*** | 1.82±0.13*** | 2.54±0.25*** | 3.12±0.30*** |
| | 60 | 4.40±0.23 | 1.52±0.07 | 0.39±0.02 | 1.53±0.09** | 1.67±0.12** | 1.69±0.18** | 1.97±0.14** |
| | 120 | 4.65±0.55 | 1.68±0.03 | 0.37±0.05 | 1.73±0.13*** | 1.81±0.18*** | 1.90±0.13*** | 1.89±0.13** |
| | 240 | 5.03±0.41* | 1.81±0.09 | 0.43±0.03 | 1.94±0.09*** | 1.71±0.13** | 1.85±0.08*** | 2.38±0.21*** |
| *N. canescens* | 41.25 | 5.47±0.12** | 1.89±0.06 | 0.48±0.01 | 2.11±0.05*** | 2.11±0.04*** | 1.83±0.03*** | 2.49±0.09*** |
| | 82.5 | 3.35±0.32 | 1.50±0.15 | 0.29±0.02 | 1.67±0.12* | 1.63±0.11*** | 1.44±0.08** | 2.18±0.10*** |
| | 165 | 4.42±0.26 | 1.51±0.09 | 0.39±0.02 | 2.19±0.31*** | 1.59±0.11*** | 1.76±0.10*** | 2.27±0.09*** |
| | 330 | 4.50±0.25 | 1.62±0.57 | 0.41±0.01 | 2.80±0.23*** | 1.47±0.11** | 1.96±0.17*** | 1.71±0.20*** |
| *C. bauchiense* | 30 | 4.87±0.48* | 1.65±0.13 | 0.42±0.03 | 1.77±0.13* | 1.83±0.09*** | 1.60±0.11** | 2.40±0.24** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 60 | 5.12±0.30** | 1.85±0.14 | 0.42±0.04 | 2.07±0.10** | 1.99±0.06*** | 1.70±0.14** | 2.71±0.43*** |
| | 120 | 3.87±0.34 | 1.50±0.04 | 0.36±0.02 | 1.94±0.42* | 1.45±0.15* | 1.43±0.20 | 2.42±0.26*** |
| | 240 | 5.25±0.29** | 1.89±0.06 | 0.48±0.02 | 2.14±0.30** | 1.75±0.23** | 1.59±0.20* | 1.56±0.07 |

Values represent Means ± s.e. m of six animals per group; *; **; ***Values significantly different at respective thresholds of $P< 0.05$, $P< 0.01$, $P< 0.001$ from those of the control-neutral group (Fisher SSD Test); diuretic action = total urine volume of treated rats/total urine volume of rats in the neutral control group; diuretic activity = diuretic action of test extract/diuretic action of standard drug; REEF= (Test urine volume X Test electrolyte concentration)/(Neutral control urine volume X Neutral control electrolyte concentration)

**Table 5:** Effects of treatments on the anion gap and indices of aldosterone secretion, thiazide and carbonic anhydrase inhibition

| Plants | Dose (mg/kg) | Anionic gap | Ratio ($HCO_3^-$/Cl) | Aldosterone secretion Index | Thiazide diuretic index | Carbonic anhydrase inhibition index |
|---|---|---|---|---|---|---|
| Control | Neutral | -256.31±10.17 | 1.79±0.24 | 6.04±0.08 | 0.34±0.04 | 2.63±0.29 |
| | Positive | -250.86±6.74 | 1.73±0.10 | 6.72±0.41 | 0.34±0.03 | 2.68±0.22 |
| *S. Veronicaefolia* | 30 | -248.33±7.56 | 1.67±0.15 | 5.13±0.17 | 0.29±0.01 | 2.90±0.09 |
| | 60 | -300.25±18.68* | 2.35±0.25 | 6.69±0.15 | 0.41±0.04 | 2.36±0.24 |
| | 120 | -303.92±5.026** | 1.83±0.19 | 7.02±0.52 | 0.35±0.03 | 2.49±0.20 |
| | 240 | -294.67±15.05* | 1.84±0.22 | 5.39±0.45 | 0.31±0.01 | 2.97±0.15 |
| *N. canescens* | 41.25 | -210.57±7.10 | 2.02±0.16 | 5.85±0.31 | 0.37±0.02 | 2.36±0.11 |
| | 82.5 | -371.75±23.90*** | 2.52±0.27** | 6.28±0.10 | 0.44±0.06 | 2.17±0.28 |
| | 165 | -288.93±26.56 | 1.89±0.17 | 5.20±0.59 | 0.32±0.01 | 2.78±0.20 |
| | 330 | -402.57±30.89*** | 1.37±0.10 | 2,84±0,15*** | 0.25±0.03 | 3.12±0.24 |
| *C. bauchiense* | 30 | -223.10±21.79 | 2.53±0.16** | 6.14±0.19 | 0.37±0.02 | 2.53±0.13 |
| | 60 | -239.15±13.33 | 1.97±0.14 | 6,14±0.15 | 0.39±0.03 | 2.44±0.20 |
| | 120 | -217.13±13.22* | 1.88±0.12 | 5.27±0.33* | 0.35±0.01 | 2.34±0.08 |
| | 240 | -232.26±9.22 | 1.75±0.10 | 3.75±0.19*** | 0.30±0.01 | 2.70±0.17 |

Values represent Means ± S.E.M of six animals per group; *; **; ***Values significantly different at respective thresholds of $P< 0.05$, $P< 0.01$, $P< 0.001$ from those of the control-neutral group (Fisher SSD Test). Aldosterone secretion index (Na+/K+); Thiazide diuretic index (Na+/Cl-); Carbonic anhydrase inhibition index [Cl-/(Na++ K+)]; Anion gap [(Na+ + K+)-Cl-]

**Anti-free radical effects of AESLSV, AESLNC and AECB**

**Diphenyl-2 picrylhydrazyl (DPPH) radical reduction test**

Figure 2 shows the scavenger activity of the different extracts on the DPPH radical expressed in percentage.

A low activity of the AESLSV is obtained, with the percentage of inhibition of 46.20% at the concentration of 200 µg/ml. The AESLNC showed activities of 58.32 and 69.84% while AESLCB showed 48.40 and 69.84% of activities respectively at concentrations 50 and 100 µg/ml.

The $IC_{50}$ (Inhibitory Concentration 50) of the different extracts were also determined and were 204.17, 29.36 and 35.30µg/mL respectively for AESLSV, AESLNC and AESLCB (Table 6).

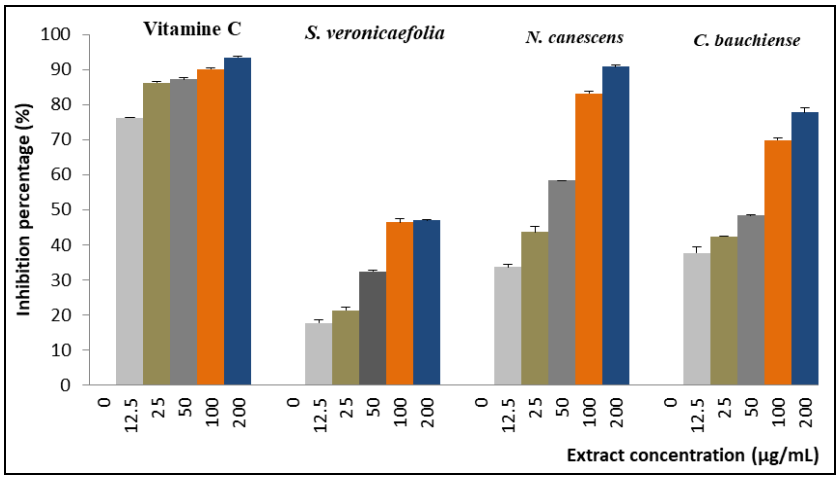


**Fig 2:** Percentage of DPPH inhibition (%) as a function of extract concentrations and ascorbic acid. Values are the mean + S.E.M of 3 replicates

**Table 6:** Inhibitory concentration 50 of the different extracts and reference molecule on DPPH

| Name of the plant or of the reference molecule | Inhibitory concentration 50 (µg/mL) |
|---|---|
| *S. veronicaefolia* | 204,17±4.69 |
| *N. canescens* | 29.36±1.03 |
| *C. bauchiense* | 35.30±1.52 |
| Vitamine C | 10.61±0.28 |

Values are the mean ±SEM of 3 replicates

**FRAP (Ferric Reducing Antioxidant Power)**

The iron reducing power of the different plant extracts studied was evaluated and the results are presented in the following figure 3. It appears that all aqueous extracts have an antioxidant potential that increases with the concentration. However, the reducing power of vitamin C is significantly different from those of all the others.

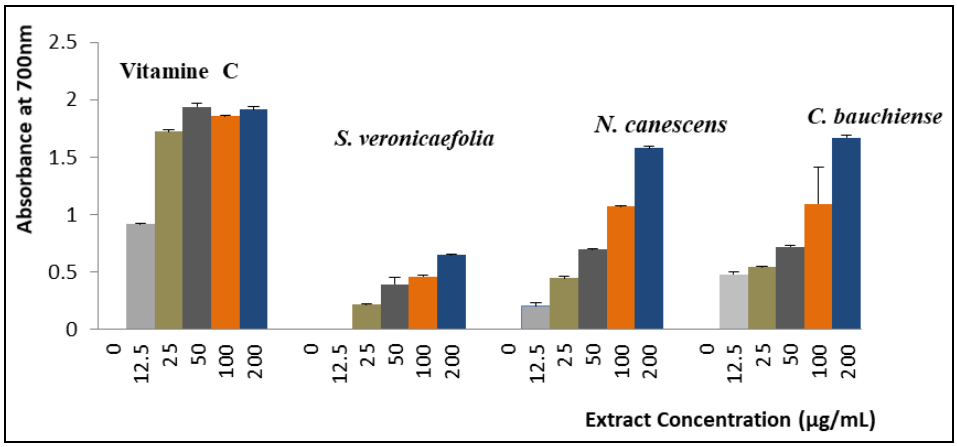


**Fig 3:** Ferric Reducing Antioxidant Power in fonction of different concentrations of each plant extract and Vitamin C; Values are the mean + S.EM of 3 replicates

## Discussion

The results of the study show that AESLSV, AESLNC and AESLCB all significantly increased the percentage of urinary excretion, the urinary levels of all electrolytes assessed and their renal excretion fractions (REEF). Urinary conductivity, which is an indirect measure of ions in the urine, also increased in several groups. The increase in sodium and water excretory activity by a xenobiotic provides a solid basis for its diuretic and anti-hypertensive action [17]. Indeed, a substance is considered diuretic when it is able to stimulate renal excretion of fluid and electrolytes [18]. Therefore, all these results show that our plant extracts have a diuretic power and justify their strong use in traditional medicine to limit the hydro-sodic retention during pregnancy.

The urinary anion gap (UAG) is used to estimate the amount of $NH_4^+$ excreted as it is very difficult to measure them routinely. When the urine is acidic, the urinary cations excreted are mainly $NH_4^+$. When the UAG is strongly negative, large amounts of urinary $NH_4^+$ are present and it reflects normal kidney function in case of excessive bicarbonate loss. Increased $NH_4^+$excretion maintains electrolyte balance. In contrast, UAG is positive in distal tubular acidosis. [19]. Therefore, the decrease in UAG observed with AESLSV and AESLNC reflects an increase in renal $NH_4^+$ excretion in response to increased bicarbonate excretion. The increase in TAU observed with AESLCB would be due to an acidification of the medium.

The diuretic activities of the three plant extracts can be considered as good because whatever the dose administered, the diuretic action is greater than or equal to 1.50. According to Abdala *et al*. in 2008 [20], the diuretic activity of a substance is good if the value of its diuretic action is higher than 1.50; moderate if this value is between 1.00 and 1.50; weak if it varies between 0.72 and 1.00. When this value is lower than 0.72, there is no diuretic activity. These diuretic activities of plant extracts can be exerted on different segments of the nephron through several types of mechanisms. In an attempt to identify the mechanisms by which these extracts would act, several diuretic indices were determined. Although no significant changes were noted with the thiazide secretion and carbonic anhydrase inhibition indices, a decrease in the aldosterone secretion index ($Na^+/K^+$) was observed with AESLNC and AESLCB. The aldosterone secretion index can be used to determine the mechanism of action of diuretics [21]. Indeed, for loop diuretics, this index is approximately equal to that of the control because they eliminate the two electrolytes ($Na^+$, $K^+$) in similar quantities. It is lower than the control for thiazides ($K^+$ excretion greater than $Na^+$); and higher than the control for hypokalemic drugs ($K^+$ excretion less than $Na^+$) [22]. This would mean that the AESLNC and AESLCB may be thiazides while AESLSV would be a loop diuretic. Also, no variation in pH was noted in all treated animals. This would prove that none of the different extracts acts as a carbonic anhydrase inhibitor. Indeed, the carbonic anhydrase inhibitors block the $Na^+/H^+$ exchangers in the proximal tubule, thus leading to a decrease in the reabsorption of bicarbonates and consequently that of the sodium associated with these anions [23].

The significant increase in bicarbonate excretion observed could be an advantage for these plant extracts since a decrease in bicarbonate levels is necessary during pregnancy to facilitate fetal $CO_2$ output and oxygen uptake [24].

Furthermore, phytochemical studies of our plant extracts have revealed the presence of flavonoids and polyphenols. It has been proven that a large number of compounds present in plants (flavonoids, alkaloids, saponins.) are responsible for their diuretic effect although their mechanism of action remains to be elucidated [25, 33]. However, it has been shown that some flavonoids exert their diuretic activity by inhibiting Na+/K+/2Cl- cotransporters [21]. Hence, the diuretic activity of the different plant extracts would be due to the presence of these secondary metabolites in them.

Considering the involvement of oxidative stress in the development of several pathologies of pregnancy with which hydro-sodium retention is associated (hypertension, pre-eclampsia.), the possibility for ours aqueous extracts of plants to have anti-radical properties would be an additional asset for their use during pregnancy. For this reason, their antioxidant potential was evaluated. Since several characteristic mechanisms and reactions are involved in the antioxidant defense system of the organism, a single test is not sufficient to conclude on the antioxidant potential of a compound [27]. By Therefore, the anti-radical potential of our different plant extracts was evaluated by different tests namely: DPPH and FRAP test.

DPPH is usually used as a substrate to evaluate the antioxidant activity of an antioxidant agent. In our studies, the different aqueous extracts showed IC50s of 204.17, 29.36 and 35.30 for AESLSV, AESLNC and AESLCB respectively. According to Souri *et al* (2008) [28], the antioxidant activity of a plant extract is high when its IC50< 20 µg/mL, moderate when 20 µg/mL ≤ IC50 ≤75 µg/mL and low when its IC50> 75 µg/mL. Hence the AESLNC and AESLCB would have moderate antioxidant activity while AESLSV would have a low activity.

At all concentrations tested, the reducing powers of the

AESLNC and AESLCB are much higher than that of the AESLSV, but much lower than that of vitamin C. These reducing powers of the different extracts would probably be due to the presence of hydroxyl groups in the phenolic compounds they contain since these extracts contain total phenols and flavonoids. Indeed, the antioxidant capacity of plants is mainly due to their richness in phenolic compounds, which are able to donate hydrogen atoms to inhibit lipid peroxidation [29]. The presence and number of free OH groups are determining factors in the antioxidant activity of polyphenols [30]. Within this family, flavonoids are those that have a protective effect on biological systems through their ability to transfer electrons to free radicals, chelate metals, activate antioxidant enzymes and inhibit oxidases [31]. Hence, our extracts in addition to having a good diuretic potential, also possess antioxidant activities.

In addition, polyphenolics are known to have venotonic and vasculoprotective properties [32]. These different properties could be beneficial in preventing vascular damage in pregnant women.

## Conclusion

The objective of this work was to evaluate the diuretic and antioxidant activities of aqueous extracts of *S. veronicaefolia*, *N. canescens* and *C. bauchiense*. The results obtained allow us to draw the following conclusion: AESLSV, AESLNC and AESLCB have diuretic and antioxidant activities. This allows us to state that the use of the three study plants in traditional medicine as diuretic is justified. Their antioxidant potential would contribute to their preventive or curative effect on hypertension during pregnancy.

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