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DIURETIC, ANTIDIURETIC AND LAXATIVE ACTIVITIES OF *ANTHOCLEISTA VOGELII* EXTRACTS IN RATS

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Abstract

Background: *Anthocleista vogelii* Planch is a medicinal plant used by traditional healers in the treatment of Malaria, hypertension, ulcer, obesity, diabetes, and as a diuretic and purgative in Africa. Therefore, the present study sought to evaluate the diuretic, antidiuretic and laxative activities of the root bark of *A. vogelii* extracts and fractions in male Sprague-Dawley rats.

Materials and methods: Sixty rats were placed in 10 groups which included: control (normal saline), furosemide/sodium picosulfate (25 mg/kg) as standard drugs, methanol extracts (250 mg/kg and 500 mg/kg), and 125 mg/kg and 250 mg/kg of saponin, flavonoid and neutral alkaloid fractions of *A. vogelii*. The drugs/extracts/fractions were administered orally using normal saline as the vehicle.

Results: The 500 mg/kg methanol extracts (ME) significantly increased ($p<0.05$) the urine volume and electrolytes (Na^+, K^+ and Cl^-) excreted, while 125 mg/kg and 250 mg/kg flavonoid fraction (FF) decreased significantly ($p<0.05$) urine volume and electrolytes (Na^+, K^+ and Cl^-) excreted, but doses of saponin fraction (SF) and neutral alkaloid fraction (NAF) had no significant difference ($p<0.05$) compared to the control after 5 hrs. ME, SF, NAF significantly increased ($p<0.05$) the fecal output of the animals when compared to the control, while FF showed no significant difference ($p<0.05$) after 8 hrs of administration.

Conclusions: This study determined that ME revealed diuretic activity, although not remarkable to furosemide, while FF showed antidiuretic activity, and potent laxative activities were discovered in ME and SF of *A. vogelii*.

Keywords: *Anthocleista vogelii*, Diuretic, Anti-Diuretic, Laxative, Traditional healers

Introduction

Diuresis refers to increased urine production and excretion by the kidneys, and sometimes it is accompanied by loss of electrolytes such as sodium, chloride and potassium. It could be caused by high blood sugar, diabetes mellitus, diabetes insipidus, medications, polydipsia, acute renal failure, etc. Some side effects of diuresis include fatigue, headache, weakness, lethargy, hypokalemia, hypomagnesemia, hyponatremia, metabolic acidosis and hypotension (Woodrow, 2002). Despite the side effect of diuresis, intentional diuresis is necessary in the treatment or management of kidney disorders, hypertension and congestive heart failure, in order to remove water from patients' body. Few examples of diuretics include furosemide, hydrochlorothiazide, mannitol, bumetanide, chlorothiazide and amiloride.

On the other hand, there are antidiuretics, which are meant to regulate body water balance by reducing urine production and excretion, thereby opposing diuresis. Although, antidiuretic hormones are found in most mammals, the use of antidiuretic agents/drugs is necessary for certain conditions such as diabetes insipidus, bed-wetting, and increased rate of urination due to head surgery or trauma. Constipation is the condition of difficulty or less frequency in bowel movements in

a person. If constipation is prolonged and severe, bowel obstruction occur leading to life threatening situations. Laxatives, such as Bisacodyl or Laxoberon, are used to treat constipation, to loosen stools and enhance bowel movement. The search for therapeutic agents from plant sources by researchers is rising because it is considered readily available, least expensive, less side effects and provides other medicinal benefits attributable to the plants.

The shrub-like tree, *Anthocleista vogelii* is commonly called cabbage tree. It is widely distributed across tropical Africa, Madagascar and the Comoros (Leeuwenberg, 1973; 1983). In Nigeria, *A. vogelii* is popularly called mpoto (Igbo), sapo (Yoruba) and kwari (Hausa) by the natives (Anyanwu et al., 2015), and it is widely spread across the southern part of the country (Edwin-Wosu et al., 2015). The traditional medicinal use of *A. vogelii* in the treatment of Malaria, hypertension, diabetes, stomach disorders, and as a diuretic and purgative has been reported (Burkill, 1985; Igoli et al., 2005; Ariwaodo et al., 2012; Gbolade, 2012; Soladoye et al., 2012; Olorunnisola et al., 2015; Anyanwu et al., 2015).

The traditional medicinal uses of *A. vogelii* have prompted the search for therapeutic agents against various diseases through several pharmacological studies on the crude extracts, fractions, and isolated chemical constituents. Several studies have shown that *A. vogelii* possess antidiabetic, antimicrobial, antiplasmodial, spasmogenic, antiulcerogenic, anti-obesity, antitrypanosomal and fertility activities (Eloff, 1998; Ateufack et al., 2007; Abu et al., 2009; Alaribe et al., 2012; Anyanwu et al., 2013; Olubomehin et al., 2013; Ateufack et al., 2014; Oladimeji et al., 2014; Anyanwu et al., 2015).

Before now, the traditional use of *A. vogelii* as diuretic and laxative was yet to be scientifically validated, therefore, our present study aimed to evaluate the diuretic, antidiuretic and laxative activities of the root bark of *A. vogelii* in male Sprague-Dawley rats.

Materials and Methods

Plant material

The root bark of *Anthocleista vogelii* Planch was collected from a farmland in Ovakali, Ngor-Okpala Local Government Area of Imo State, Nigeria. The plant was authenticated by Ihuma, J. O., Department of Biological Sciences, Bingham University, Nigeria and *Anthocleista vogelii* Planch (GA134-7421) specimens were deposited.

Preparation of methanol extract

The harvested root bark of *A. vogelii* were cut into smaller pieces, dried at room temperature and milled into coarse powder. One kilogram of the powder was extracted with n-hexane for 12 hrs, and then with methanol by maceration for 72 hrs at room temperature and both processes was repeated three times. Filtration was first done by muslin cloth and then Whattman filter paper before the methanol filtrate was concentrated under reduced pressure at 40^{o}C using a rotary evaporator. The percentage yield of the methanol extract was 14.8%.

Preparation of fractions

Preparation of saponin fraction

The saponin fraction was prepared by the method described by Hostettmann et al. (1991) as reported by Sarker et al. (2005). The concentrated defatted methanol extract (75g) was extracted with water and partitioned with n-butanol (3x500ml). The n-butanol partition was separated from the aqueous partition using separating funnel. Diethyl ether was added to the n-butanol partition to precipitate crude saponin mixture which was collected after decantation and centrifugation. The percentage yield of the saponin fraction was 20.25%.

Preparation of flavonoid fraction

Powdered plant material was extracted with 80% methanol: 20% water and the process were repeated three times. The combined filtrate was concentrated to 1/10th of original volume, which was acidified by adding 2 M H_2SO_4 to facilitate the extractability of flavonoids (which are acidic in nature) and partitioned with chloroform three times as described by Harborne (1998). The chloroform layer was pooled together and concentrated under reduced pressure to yield 9.2% of flavonoid rich fraction.

Preparation of neutral alkaloid fraction

The neutral alkaloid fraction was obtained by the method described by Cordell (1981) as reported by Sarker et al. (2005), and slight modifications were made in accordance to our laboratory procedures. Fifty grams of the concentrated defatted methanol extract was extracted with 1N H_2SO_4 for 3 times. The acid extracts were combined and basified using ammonia to neutral pH (7.0) in an ice cold bath, and then it was partitioned with ethyl acetate. The ethyl acetate partition

was separated from the aqueous partition by a separating funnel and the former was concentrated using rotary evaporator to give the neutral alkaloid fraction. The percentage yield of the alkaloid fraction was 7.9%.

Reference drugs

Lasix (furosemide) manufactured by Sanofi-aventis Pakistan Limited was used as the diuretic reference drug. Laxoberon (sodium picosulfate) manufactured Merck (Private) Limited, Pakistan under the license of Boehringer Ingelheim Pharma, Germany was used as the laxative reference drug.

Experimental animals

Sixty male Sprague–Dawley rats (180 ± 10 g) purchased from the National Institute of Health, Islamabad, Pakistan were kept in cages at the Animal House of Pharmacy Department, COMSATS Institute of Information Technology, Abbottabad, Pakistan. The rats were left to acclimatize under controlled temperature (23 ± 2°C) and 12:12 h light/dark cycle with water and food *ad libitum*. All experimental procedures followed the European Community guidelines (EEC Directive of 1986; 86/609/EEC) for animal use and that of the Research Ethics Committee of Pharmacy Department, CIIT, Abbottabad which gave the ethical approval number (PHM-0024/EC/M-4-5.15).

Administration of drug/extracts/fractions

The drug/extracts/fractions were dissolved in normal saline as the vehicle and using an oral gavage attached to a graduated syringe, the rats were administered the doses according to their groups.

Diuretic activity

The animals were separated into 10 groups of 6 rats per group and diuretic activity was performed according to the method described by Lipschitz et al. (1943) with slight modification by Murugesan et al. (2000). The animals were deprived of food and water for 18hrs prior to the experiment. Animals in Group 1 served as negative control, received normal saline (25 mL/kg); Group 2 received the standard drug, furosemide (25 mg/kg); Groups 3 and 4 received 250 mg/kg and 500 mg/kg *A. vogelii* methanol extract respectively; Groups 5 to10 received 125 mg/kg and 250 mg/kg of *A. vogelii* fractions as indicated in Table 1. The drug/extracts/fractions were administered orally using normal saline as the vehicle.

After administration, immediately the rats were placed in metabolic cages (two rats per cage) designed to separate urine and fecal matter. The urine volume was measured at 5 hrs post administration, and during this period no water or food was given to the animals. Urinary electrolytes concentrations, Na^+, K^+ and Cl^- were estimated.

Determination of urinary electrolyte concentrations

Urine samples were prepared by the conventional digestion method described by Panhwar et al. (2014). Exactly 0.5 mL of urine was placed in 50 mL flasks, and 5 mL of concentrated HNO_3-H_2O_2 (2:1, v/v) was added and then heated on an electric hot plate for about 3 hrs at 80^OC until a clear transparent digest was obtained. Thereafter, the final solution was topped to 10 mL with 2M HNO_3 and a portion placed in glass vials from where it was aspirated and analyzed for Na^+ and K^+ using flame by the AAnalyst 700 Perkin Elmer Atomic Absorption Spectrophotometer. The concentrations of Na and K^+ for each urine sample were displayed underneath the calibration graphs for Na^+ and K^+ respectively on the computer screen attached to the AAS.

Chloride ion concentration was determined by titration using Mohr's method as reported by Tripathi and Govil (2001) with slight modifications. Exactly 1 mL of urine sample was diluted with 20 mL of distilled water in a flask. Then, 1 mL potassium chromate indicator (5%) was added to it and titrated with 0.02N silver nitrate till the first appearance of red-brown colour of the solution was obtained.

Chloride (mg/l) = $(V_s - V_b)$ x N x 35.5 x 1000/ ml sample

(V_s = volume of $AgNO_3$ used for sample titration; V_b= volume of $AgNO_3$ used for blank titration; N = Normality of $AgNO_3$).

Laxative activity

The laxative activity was performed according to the method reported by Capasso et al. (1986), after a wash out period of one week from the previous experiment. The male rats were fasted for 12 hrs prior to the experiment, but water was made available *ad libitum*. The rats were placed into 10 groups of 6 rats per group. The animals received sodium picosulfate (25 mg/kg) or different doses of extracts/fractions of *A. vogelii* orally as in the previous experiment using

normal saline as the vehicle (Fig. 2). After administration, the rats were placed in cages suitable for collection of feces which was collected and weighed at the 8th hr.

Statistical analysis

The data obtained from the experiments were expressed as Mean ± S.E.M. Statistical significance of difference in values between groups was analyzed by one-way ANOVA using GraphPad Prism followed by Dunnett's multiple comparison tests. The level of significance was P<0.05.

Results

Diuretic studies

Urine volume excretion after 5 hrs

The urine excretion induced by the methanol extract (ME) of *A. vogelii* was dose dependent, however only 500 mg/kg of ME revealed slight significant increase ($p < 0.05$) in the volume of urine excreted when compared with the control (Fig. 1). The volume of urine excreted by furosemide group was more than twice the volume of urine excreted by the control and all the extracts/fractions of *A. vogelii*. As seen in Fig. 1, the 125 mg/kg and 250 mg/kg doses of saponin fraction (SF) and neutral alkaloid fraction (NAF) showed no significant difference ($p < 0.05$) in the urine volume excretion compared to the control. However, the 125 mg/kg and 250 mg/kg doses of flavonoid fraction (FF) significantly decreased the urine output of the animals.

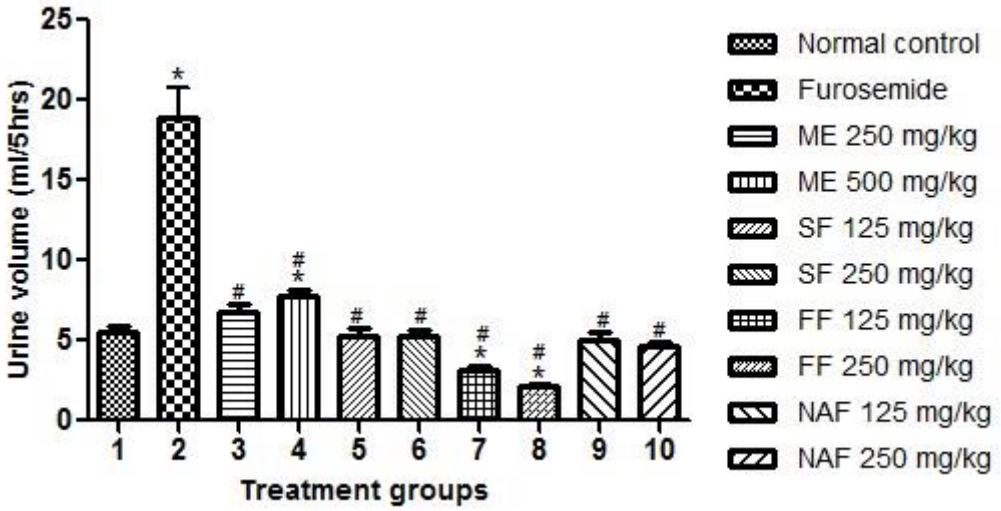

Figure 1: Urine volume of the animals after 5 hrs of extract/drug administration
Values are expressed as means ± SEM, * significantly different ($p < 0.05$) from control, # significantly different ($p < 0.05$) from furosemide.

Urinary electrolyte excretion after 5 hrs

The results of the urine electrolytes of all groups after 5 hrs of oral administration were shown on Table 1. The concentration of Na^+, K^+ and Cl^- found in the urine of the rats treated with 500 mg/kg of ME and furosemide was significantly increased compared to the control. The 125 mg/kg and 250 mg/kg of FF showed significant decrease ($p < 0.05$) in the concentration of Na^+, K^+ and Cl^- found in the urine, while the doses of SF and NAF had no significant difference. The concentration of Na^+, K^+ and Cl^- in the excreted urine as induced by furosemide were significantly increased ($p < 0.05$) compared to the different doses of extracts/fractions of *A. vogelii*. The 250 mg/kg of ME showed significantly increased ($p < 0.05$) Cl^- concentration in the urine (Table 1).

Table 1: Diuretic activity of *A. vogelii* extracts/fractions in rats after 5 hrs of oral administration

Group	Dose (mg/kg)	Diuretic index	Urine Electrolyte Concentration (mEq/L/5hrs)			Saluretic index			Na^+/K^+ ratio
			Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	
Normal Control		1	55.28 ± 4.17	25.65 ± 1.95	232.59 ± 20.94	-	-	-	2.16
Furosemide	25	3.40	126.96 ± 22.59*	64.14 ± 6.58*	538.60 ± 57.59*	2.30	2.50	2.32	1.98
Methanol Extract	250	1.22	68.68 ± 4.35#	31.33 ± 1.97#	368.38 ± 22.31*#	1.24	1.22	1.58	2.19
	500	1.40	78.13 ± 4.10*#	36.07 ± 1.94*#	433.51 ± 19.93*#	1.41	1.41	1.86	2.17
Saponin Fraction	125	0.95	52.61 ± 4.62#	24.41 ± 2.10#	222.84 ± 16.61#	0.95	0.95	0.96	2.16
	250	0.95	52.61 ± 3.49#	24.44 ± 1.63#	233.41 ± 19.84#	0.95	0.95	1.00	2.15
Flavonoid Fraction	125	0.56	31.18 ± 3.42*#	14.31 ± 1.62*#	135.98 ± 18.73*#	0.56	0.56	0.58	2.18
	250	0.39	21.46 ± 1.70*#	9.95 ± 0.84*#	93.26 ± 5.32*#	0.39	0.39	0.40	2.16
N. Alkaloid Fraction	125	0.89	49.46 ± 5.04#	22.93 ± 2.32#	207.66 ± 22.03#	0.89	0.89	0.89	2.16
	250	0.83	46.38 ± 2.12#	21.36 ± 1.10#	202.01 ± 13.95#	0.84	0.83	0.87	2.17

Values are expressed as means ± SEM, *significantly different ($p < 0.05$) from control, #significantly different ($p < 0.05$) from furosemide. Saluretic index = mEq test group / mEq control group. Diuretic index = volume problem group/volume control group.

Fecal output after 8 hrs

Figure 2 showed the fecal out of the animals after 8 hrs of extract/fractions administration. The feces produced by the ME and SF were dose –dependent, as 500 mg/kg of ME, 125 mg/kg and 250 mg/kg of SF significantly increased ($p < 0.05$) the fecal output of the animals more than the control and sodium picosulfate groups. Sodium picosulfate significantly increased ($p < 0.05$) the fecal output compared to the control. Also, both doses of NAF significantly increased ($p < 0.05$) the feces output compared to the control, but their effect was not significantly different from sodium picosulfate. There was no significant difference between the fecal output of both doses of FF and the control (Fig. 2).

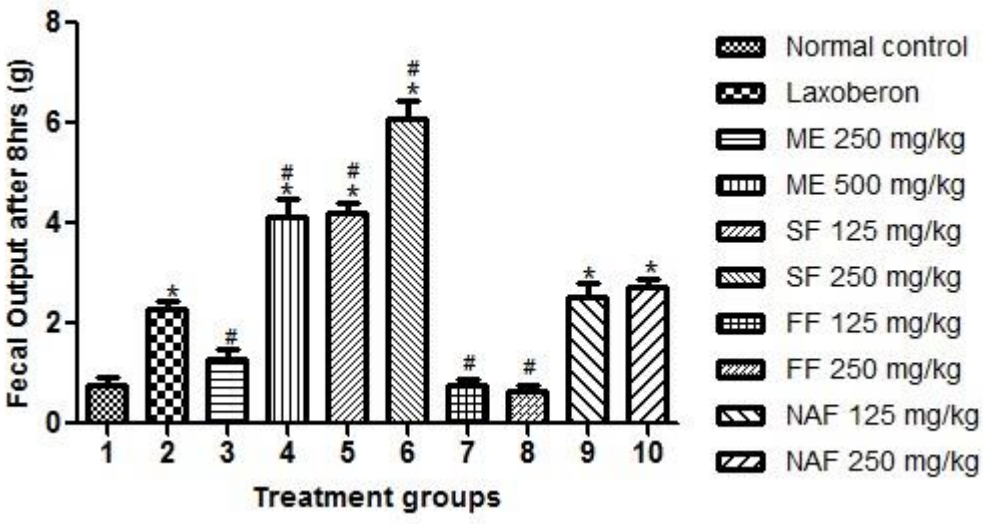

Figure 2: Fecal out of the animals after 8 hrs of extract/drug administration
Values are expressed as means ± SEM, * significantly different ($p < 0.05$) from control, #significantly different ($p < 0.05$) from Laxoberon (sodium picosulfate).

Discussion

This study revealed that only the methanol extracts (ME) of *A. vogelii* have a dose-dependent diuretic activity. Furosemide, a loop diuretic, increased the excretion of water, sodium and potassium which was expected and also observed in this study. Although the values of urine and electrolytes (Na^+, K^+ and Cl^-) excreted by 500 mg/kg of ME was not close to those of furosemide, the significant increase compared to the control indicates possible diuretic activity. The values of urine and electrolytes of SF and NAF were similar to those of the control, thus both fractions showed no diuretic activity indicating the absence of diuretic compounds in them. Therefore, among the tested extracts/fractions of *A. vogelii*, only ME revealed diuretic activity.

However, it is not certain if the diuretic activity of the ME was due to presence of diuretic compounds or a synergism in the various bioactive compounds of the plant; as its possible for a plant extract to manifest combined effect of several compounds and/or due to secondary metabolite (Tanira et al., 1988). So, it is necessary to determine the diuretic effect of the non-polar fraction (n-hexane fraction) and more polar fractions (tertiary and quaternary alkaloidal fractions) of *A. vogelii*.

The values of urine volume and electrolytes (Na^+, K^+ and Cl^-) of FF were significantly decreased compared to the control, which indicated an antidiuretic effect. Antidiuretic agents are known to enhance body water retention by reducing urine production and/or excretion, thereby opposing diuresis. The FF acted in an opposite manner to furosemide. While furosemide inhibits electrolyte (Na^+ and K^+) reabsorption in the thick, ascending limb of the loop of Henle (Johnson et al., 1999), FF seemed to have activated electrolyte reabsorption. The rich flavonoid content of FF suggests that flavonoids found in *A. vogelii* are responsible for its antidiuretic activity. Until now, antidiuretics have not been scientifically proven in the *Anthocleista* genus; however, *A. djalonensis* and not *A. vogelii* had been reported to be traditionally used as an antidiuretic (Lawal et al., 2010). From the findings of this study, the plant *A. vogelii* seemed to possess both diuretic and antidiuretic activities, and the discovery of plant compounds/extracts with such dual effect either from a single compound or different compounds within a plant has been reported (Chen et al., 2014; Feng et al., 2014).

The Na^+/K^+ ratios were similar among the drug, extracts or fractions and within the groups. According to Vogel (2006), values of Na^+/K^+ ratio greater than 2 indicates favourable natriuretic activity while values greater than 10 indicates

potassium sparing effect of diuretic agents in male wistar rats. Thus, it is suggested that ME of *A. vogelii* which showed diuretic effect also possessed favourable natriuretic activity as the Na^+/K^+ ratio was little above 2 (Table 1).

The increased fecal output by ME, NAF and SF shows that they have the ability to increase bowel movement and loosen feces, a characteristic of laxatives or purgatives. But only ME and SF significantly increased fecal output compared to the laxative reference drug, Laxoberon (sodium picosulfate), which indicates potent laxative activity. Since SF is rich in saponins, it is suggestive that saponins in *A. vogelii* are responsible for its laxative activity.

Based on careful observation throughout the experiment, the feces of the control were black, long shape and uniform in texture, while that of ME and SF was a mix of black, brown, mushy, pasty or drawing, uniform and non-uniform in texture. This indicates that the laxative effect of ME and SF might be helpful to increase the frequency of bowel movements, increase quantity of stools, and reduce pain in the process of excretion. Thus, the results give credence to the traditional use of the plants as laxatives or purgatives.

This study determined that ME revealed diuretic activity, although not remarkable to furosemide, while FF showed antidiuretic activity, and laxative activities were discovered in ME and SF of *A. vogelii*. Further research is on-going to identify the bioactive compounds eliciting the diuretic, antidiuretic and potent laxative activities in *A. vogelii* which will be helpful towards clinical applications.

Conflict of Interest: The authors have declared that there is no conflict of interest.

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Author contributions

Prof. Chukwu E. Onyeneke designed the work on diuretic studies, Dr. Khalid Rauf designed the laxative studies, Gabriel O. Anyanwu carried out the experimental work on both diuretic and laxative studies, Ofoha Patricia Chioma and Dr. Usunobun Usunomena participated in the plant extraction/fractionation and write up, and Prof. Nisar-ur-Rehman supervised and edited the work. All authors read and approved the work.

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