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Laxative and diuretic effects of *Anchomanes difformis* (Araceae)

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Abstract

Purpose: *Anchomanes difformis* (Araceae) is widely used in subsaharan Africa against, constipation, edema, high blood pressure and anuria. The present study is undertaken to investigate laxative and diuretic activities of aqueous and ethanol extracts in normal and constipated rats.

Method: Aqueous and ethanolic leaves and rhizome extracts were orally administered in rats at doses of 400 and 800 mg/kg. Castor oil (10 ml/kg) and furosemide (10 mg/kg) were used as positive control in laxative and diuretic studies respectively. The laxative and diuretic effects were determined by measuring latency time, frequency and total volume excreted respectively of feces and urine. The physical state of the feces was also examined.

Result: Extracts of the rhizome (400 and 800 mg/kg) showed highly significant differences on the latency, the frequency and the total quantities of urine and fecal excretion in normal and constipated rats. At 800 mg/kg these effects (***) $p < 0.001$, are higher than castor oil (** $p < 0.01$) and furosemide (** $p < 0.01$). At doses of 400 (** $p < 0.5$) and 800 mg/kg (** $p < 0.01$) aqueous extract of the leaves showed significant different on the total mass of feces, and on all other parameters of urinary excretion in normal rats. There is no direct or indirect effect of the extracts on the physical characteristics of fecal excretion. The highest diuretics index of furosemide and extracts from the rhizome and leaves are respectively 1.94; 2.35 and 1.74. Lipschitz gains Showed 120% and 90% of diuretic activity for the rhizome and the leaves extracts respectively. The chemical screening confirms the presence of several metabolites.

Conclusion: *Anchomanes difformis* has interesting laxative and diuretic effects related to its use in traditional medicine. Its laxative effect would be a mechanism that passes through a stimulation of intestinal motility. Its numerous metabolites explain these effects.

Keywords: *Anchomanes difformis*, phytochemistry, pharmacology

1. Introduction

The virtues of medicinal plants are numerous and well established, they find their use in the pharmaceutical industry and in traditional medicine (Nsonde Ntandou *et al.*, 2015; 2016) [15, 16]. In Congo the cost of modern pharmaceutical drugs is not accessible to all social classes in urban areas as well as in rural areas. *Anchomanes difformis* is in the family of Araceae (Abe and Lajide, 2014) [2]. It is used in traditional medicine in several sub-saharan countries (Bouquet, 1969; Ataman and MacDonald, 2015) [7, 10]. The rhizome is used as aqueous decoction or macerated in the treatment of gonorrhoea, women abdominal pain, hernia, edema, constipation, anuria, stomach burns, tuberculosis, tachycardia, sleeping sickness, painful menstruation, prostatitis, rheumatism, scabies, colic, asthma attacks, vomiting, diarrhoea, calculations, filariasis, madness, ophthalmia in children, vertigo, leprosy, epilepsy buboes, and diabetes (Nkeoua, 1999; Fézan H. *et al.*, 2008; Joanne *et al.*, 2009; Bedigian & Adetula, 2004; Kerharo and Bouquet, 1950; and Njike Akah, 1990) [11, 6, 4]. The aqueous extract of the rhizome promotes the placenta and has lactogenic and aphrodisiac virtues (Moulero, 1975; Nguekam Wambe, 2010).

For its richness in carbohydrates, the tuber of *Anchomanes difformis* is used as a starchy food, but this requires a long period with several boiling by changing water every time to remove certain toxic substances which nature is not yet reported (Moulero, 1975).

On the phytochemical constituents, the rhizome contains numerous primary and secondary metabolites: carbohydrates, proteins, minerals, fat and amino acids; tannins, cardiac glycosides, saponins, alkaloids, flavonoids, phlebotanins, steroids, terpenoids. (Abah and al, 2011, Eke Ifeanyi *et al.*, 2013, Busson, 1963; Oyetayo, 2007) [1, 5].

The crude extract of rhizome showed renal toxicity with excessive consumption in wistar rats subacute toxicity in general guinea pigs (Tchiakpe *et al.*, 1980) [24], and gastroprotective effect (Stephen *et al.*, 2011).

A promising analgesic, anti-inflammatory, antibacterial, antipyretic, local anesthetic and intestinal myostimulant motility effects of its rhizome and leaves have already been demonstrated (Adebayo *et al.*, 2014) [3]. The intestinal myostimulant motility activity explains a potential mechanism of action of a possible laxative activity *in vitro* (Bekro *et al.*, 2012) [14]. But, we have not found an illustrative study of this laxative effect on a living animal body.

Constipation is concerned with one person in five in the world (Nguea, 2012). In Africa, the disease is one of the taboo, and often remains "secret" because of his troublesome character (Nguea, 2012). Laxatives are drugs used against the constipation related problems by increasing intestinal motility. However, modern laxative drug is limited due to the insufficient efficacy or side effects and cost (Washabau, 2003) [28]. This justified the necessity to discover and develop new laxative.

Diuretics relieve nasal congestion and peripheral edema. They reduce plasma volume and thereby venous return to the heart (Whitworth *et al.*, 2005) [29]. They are used as first-line treatment of high blood pressure associated with a non-diet soda in the treatment of renal syndromes and liver cirrhosis (Howland *et al.*, 2006; Muhammad *et al.*, 2014) [16]. Some diuretics, such as thiazides, are associated with many side effects such as hyperuricemia, acute hypovolemia, potassium loss, hypo-magnesium, hyponatremia, hypercalcemia, hyperglycemia, hyperlipidemia and hypersensitivity (Muhammad *et al.*, 2014) [16]. There is therefore an urgent need to develop new effective and better tolerated diuretic.

In Congo, several plant species are used against constipation or as diuretic in traditional medicine, but most of these species have not yet been subject of a phytochemical and ethnopharmacological validation. This study aims to realize a chemical screening and to evaluate laxative and diuretic effects of *Anchomanes difformis* (Araceae).

II. Materials and Methods

1. Plant material

Leaves and roots of plant sample were collected in May 2013 in Makana 2 village in Pool region, Republic of Congo, under the supervision of a health tradipractioner perfectly knowing the village and the plant. The sample was identified as *Anchomanes difformis* by Dr Kami botanist Botanical Laboratory of the Centre d'Etudes sur les Ressources Végétales (CERVE) in Brazzaville, Congo. A voucher specimen was deposited at the National Herbarium and registered under the number ADA005/2013.

2. Animal material

Wistar rats (males and females), weighing between 150 and 238 g were used. They were bred at the pet of the Faculty of Science and Technology at the University Marien NGOUABI, under standard conditions (25 ± 5 °C, 40-70% relative humidity and 12 h diurnal cycle light/12h darkness). They had free access to standard food and water. The ethical rules of animal experiments published by the International Association for the Study of Pain was respected (Zimmermann, 1983) [30]. One week before the experiment the animals were placed in individual metabolic cages daily, for 8 hours/day for acclimatization to experimental conditions.

3. Preparation of extracts

3.1. Aqueous extracts

Dried and powdered leaves (50 g) and rhizome (50g) materials of *Anchomanes difformis* were each put into 500 ml

of distilled water and boiling for 30 min. After cooling, the solutions obtained are filtered and the filtrates were concentrated and kept at 4 °C. Yields of extraction from the leaves and rhizome were 12% and 10% respectively.

3.2. Ethanol extract

Dried and powdered leaves (50 g) and rhizome (50g) materials of *Anchomanes difformis* were each macerated three times in 500 mL of ethanol 90 °. The resulting solution was filtered and concentrated at room temperature. The extract obtained was kept at 4 °C. The extraction yield was 8%.

4. Chemical Screening

The identification of the different chemical groups in each organ of the plant was made using conventional phytochemical characterization tests (Abe *et al.*, 2014) [2]

5. Laxative effect

On normal or on constipated rats, the test was conducted according to the method described by Mascolo *et al.*, (1994) [15]. Rats fasted twelve (12) hours prior to testing are placed in individual cages lined with filter paper and then divided into six lots of five (5) animals each. All products were administered orally. In the first lot considered as a control, animals received distilled water at a dose of 10 ml/kg body weight. In the second lot, which is the reference, the animals were treated with castor oil at a dose of 10 ml/kg body weight. Lots 3 and 4 were treated with aqueous rhizome extract (AER) respectively at doses of 400 and 800 mg/kg. While lots 5 and 6 were treated with the aqueous extract of the leaves (AEL) respectively at doses of 400 and 800 mg/kg. And lots 7 and 8 were treated with rhizome ethanol extract (EER) respectively at doses of 400 and 800 mg/kg. Observations have been done first for 8 h and then at 16th hour from the time of administration of the products. The following parameters were evaluated: latency time of the first feces excretion, frequency of fecal excretion and fecal mass eliminated of the animal. The physical properties of feces, particularly hardness, flow, color and odor were also assessed. But on loperamide constipated rat 1 h after administration of the products, all the animals were treated orally with 5 mg/kg of loperamide.

6. Diuretic effect

The method described by Lipschitz *et al.*, 1943, amended, was used. The rats maintained and treated in the same manner as above were used. The difference is that these animals were not constipated and that the reference product used in this latter experiment is furosemide at 10 mg/kg administered orally. The dose volume of solvent (distilled water) ingested by each rat was 20 ml/kg body weight. Immediately after drug administration, each animal was placed in a metabolic cage designed to separately collect urine and feces. The animals were observed for 16 h to measure the time to onset of first urine, the urinary frequency emission and the total urinary excretion. Throughout the experience, the animals do not have access to water and food. Diuretic and Lipschitz index values were calculated by the following formulas:

Diuretic index = V_t / V_c and $V_t = \text{Lipschitz value} / V_r$

V_t : average of urine volume of test lot;

V_c : average of urine volume of control lot,

V_r : average of urine volume of reference lot.

7. Statistical Analysis

The results obtained are expressed as mean \pm DSM for a number of 5 animals per group using the Microsoft Excel software Windows 7. The results obtained in the test groups were compared to the negative control group using Student's t test and significance was established with probabilities * $p < 0.5$, ** $p < 0.01$ and *** $p < 0.001$.

III. Results

1. Phytochemical profile

Anchomanes difformis extracts phytochemicals preliminary tests revealed the presence of alkaloids, saponins, flavonoids and tannins, while anthraquinones and coumarins were missing.

2. Laxative activity

In normal rats *Anchomanes difformis* showed a significant laxative effect. Its ethanolic extract of the rhizome showed, on the one hand, a significant difference (*** $p < 0.001$) on the latency of the first feces excretions at doses of 400 and 800 mg/kg (Figure 1); and on the other hand, a significant difference (* $p < 0.05$) on the feces excretion frequency (Figure 2); and a significant difference (*** $p < 0.001$) on the the total fecal mass excreted (Figure 3), at a dose of 800 mg/kg. The aqueous extract of the leaves did not show significant difference of the total quantity of feces excreted at the doses of 400 and 800 mg/kg (Figure 3). While the aqueous extract of the rhizome showed significant differences both on the fecal matter excretion frequency (* $p < 0.05$), and on the total mass of excreted feces (* $p < 0.01$).

3. Laxative activity on loperamide induced constipation

In the rats constipated by loperamide leaves and barks *Anchomanes difformis* extracts showed a significant laxative effect. The aqueous extracts of the leaves (400 mg/kg) and the rhizome (800 mg/kg), and the ethanol extract of the rhizome (800 mg/kg) showed a significant difference (*** $p < 0.001$), on time latency of the first feces excretions (Figure 4). On the feces rate excretion, 400 mg/kg, of aqueous and ethanolic extracts of leaves and rhizome showed a significant difference (* $p < 0.05$). At 800 mg/kg, aqueous and ethanolic extracts also showed a significant difference (* $p < 0.001$), on the frequency of excretion of feces. Only the aqueous and ethanolic extracts of rhizome, 800 mg/kg showed a significant difference (* $p < 0.001$), on the total mass of feces excreted. In animals treated with extracts all the stools are bacilliform, black yellow and gray, with normal odor (Table II).

4. Diuretic activity

The results of the study of the diuretic activity are shown in Figures 7 and 8 and in Table III. The aqueous extracts of the leaves (** $p < 0.01$) and the rhizome (** $p < 0.001$) at doses of 400 mg/kg and 800 mg/kg showed a significant difference in the latency of the first urinary excretions (Figure 7). The aqueous extract of the rhizome showed biggest difference significant (** $p < 0.001$), on the frequency of urinary excretions (Figure 8). At 800 mg/kg, aqueous extracts of the leaves and the rhizome also showed a significant difference on the total urine excreted (Table III).

Table I: Secondary metabolites of *Anchomanes difformis*

Extracts	Chemicals groups						
	Alkaloids	Cardiotonic heterosids	Tanins	Anthocyanins	Steroids/ terpénoids	Saponin	Flavonoïds
AER	++	+++	++	+++	+	+++	++
AEL	++	+	-	+	+	-	++

Table II: Physical characteristics of feces of animals treated with *Anchomanes difformis* leaves and rhizome extracts

AER : aqueous extract of rhizome, AEL : aqueous extract of leaves, EEL: ethanolic extract of leaves

Products	Form	physical criteria		
		Hardness	Color	Odor
Distilled water	Bacilliform	Hard (with or without loperamide)	Black	Normal
Castor oil	Amorphous	Pasty or diarrhea (without loperamide)	Black	Nauseating
		soft (with loperamide)	Yellow	Nauseating
AEL	Bacilliform	Hard (with or without loperamide)	Black	Normal
EEL	Bacilliform	Hard (with or without loperamide)	Black	Normal
AER	Bacilliform	Hard or soft (with or without loperamide)	Black yellow and grey	Normal
EER	Bacilliform	Hard or soft (with or without loperamide)	Black yellow and grey	Normal

Table III: Effect of *Anchomanes difformis* leaves and rhizome aqueous extracts on urinary volume excreted in rats.

AER : aqueous extract of rhizome, AEL : aqueous extract of leaves

Treatment	Dose (mg/kg)	Urine volume in mL/100g/8h	Diuretic index	Lipschitz value
Distilled water	20 ml/kg	3,60 \pm 0,15		
Furosemide	10 mg/kg	6,98 \pm 0,18**	1,94	
AEL	400 mg/kg	5,50 \pm 0,17 *	1,52	0,79
AER	400 mg/kg	5,60 \pm 0,17*	1,56	0,8
AEL	800 mg/kg	6,27 \pm 0,20**	1,74	0,9
AER	800 mg/kg	8,50 \pm 0,26***	2,36	1,22

Values shown are mean \pm S.E.M (n = 5) and the values are compared with control group and considered significant at *** $p < 0.001$; ** $p < 0.01$ and * $p < 0.05$.

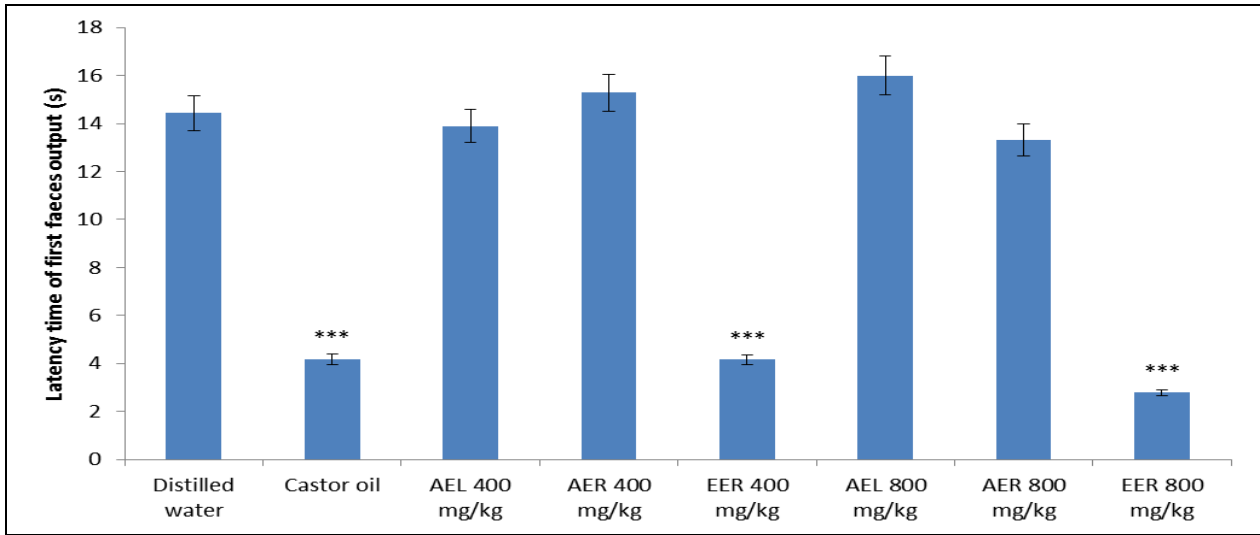


Fig 1: Effect of *Anchomanes difformis* leaves and rhizome on latency time of faeces output in normal rats. Values given are mean \pm S.E.M of five observations, ** $p < 0.01$ and *** $p < 0.001$ compared with the control group. AER : aqueous extract of rhizome, AEL : aqueous extract of leaves, EER: ethanolic extract of rhizome.

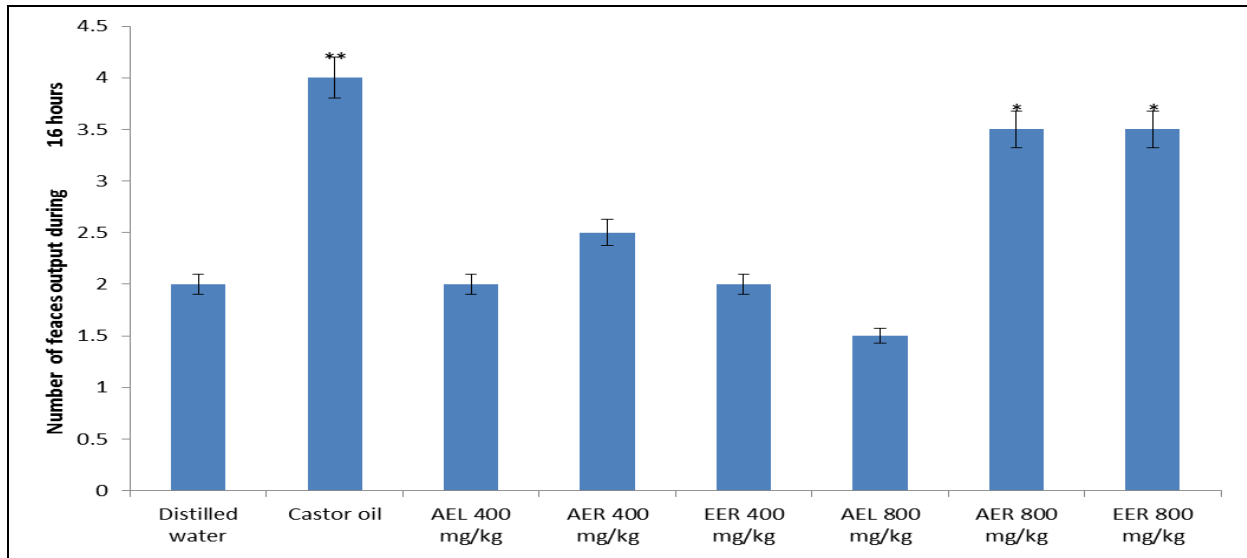


Fig 2: Effect of *Anchomanes difformis* leaves and rhizome on frequency of faeces output in none constipated rats. Values shown are mean \pm S.E.M (n = 5) and the values are compared with control group and considered significant at ** $p < 0.01$ and * $p < 0.05$. AER : aqueous extract of rhizome, AEL : aqueous extract of leaves, EER: ethanolic extract of rhizome

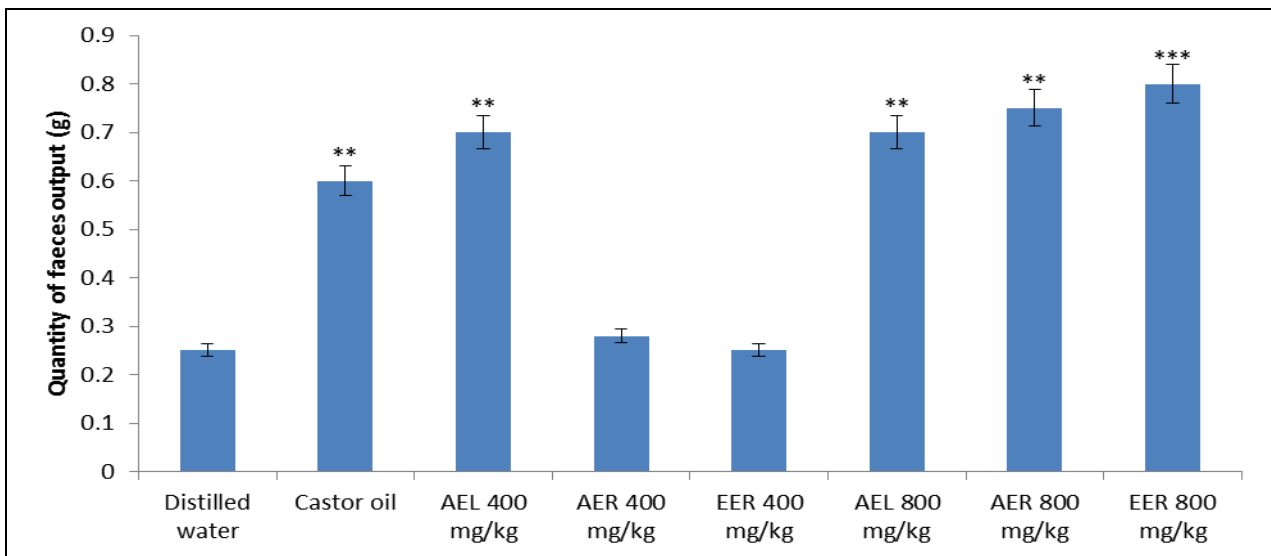


Fig 3: Effect of *Anchomanes difformis* leaves and rhizome on total quantity of faeces output in normal rats. Values given are mean \pm S.E.M of five observations, ** $p < 0.01$ and *** $p < 0.001$ compared with the control group. AER : aqueous extract of rhizome, AEL : aqueous extract of leaves, EER: ethanolic extract of rhizome

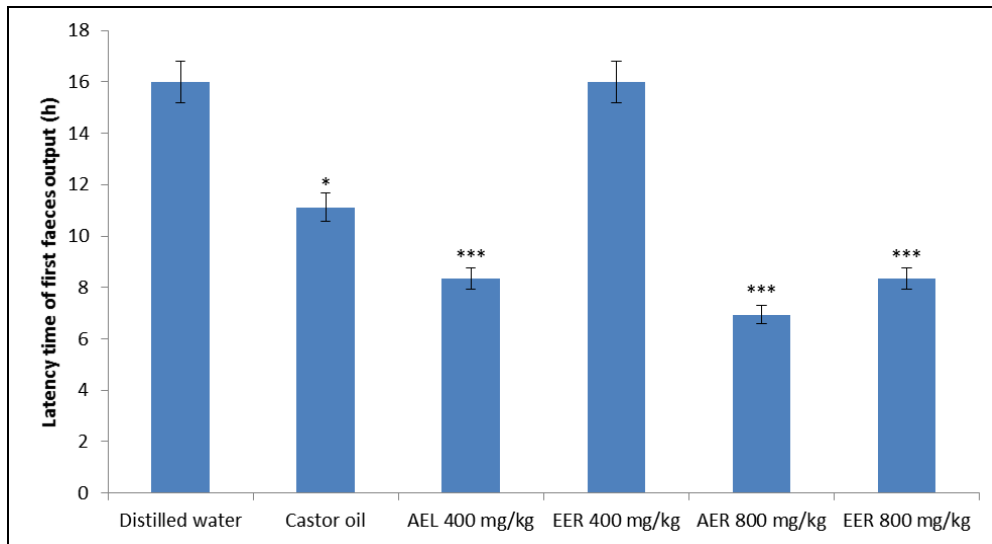


Fig 4: Effect of *Anchomanes difformis* leaves and rhizome on latency time of faeces output on loperamide induced constipation rats. Values given are mean \pm S.E.M of five observations, *** p < 0.01 and * p < 0.05 compared with the control group. AER : aqueous extract of rhizome, AEL : aqueous extract of leaves

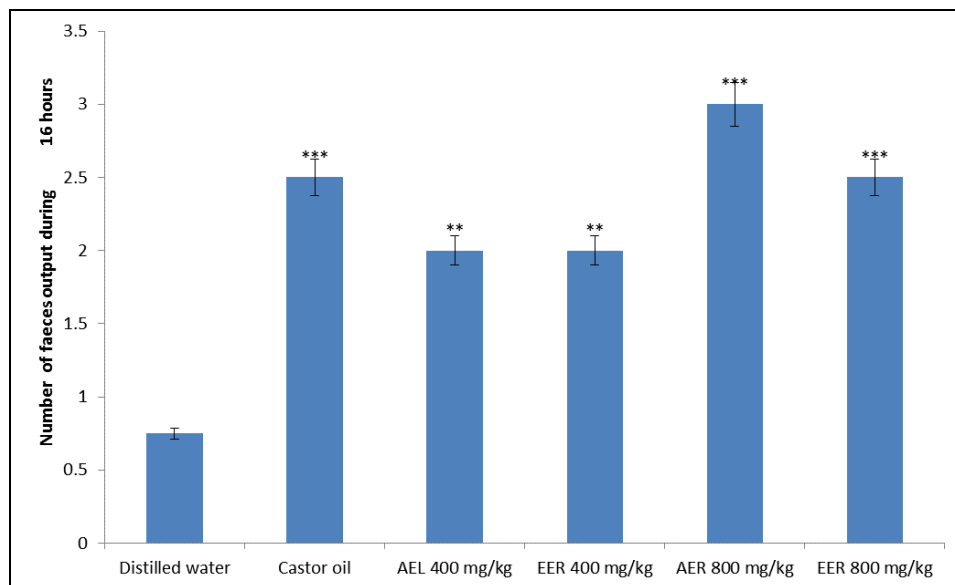


Fig 5: Effect of *Anchomanes difformis* leaves and rhizome on frequency of faeces output on loperamide induced constipation rats. Values given are mean \pm S.E.M of five observations, *** p < 0.01 and ** p < 0.01 compared with the control group. AER : aqueous extract of rhizome, AEL : aqueous extract of leaves, EER: ethanolic extract of rhizome

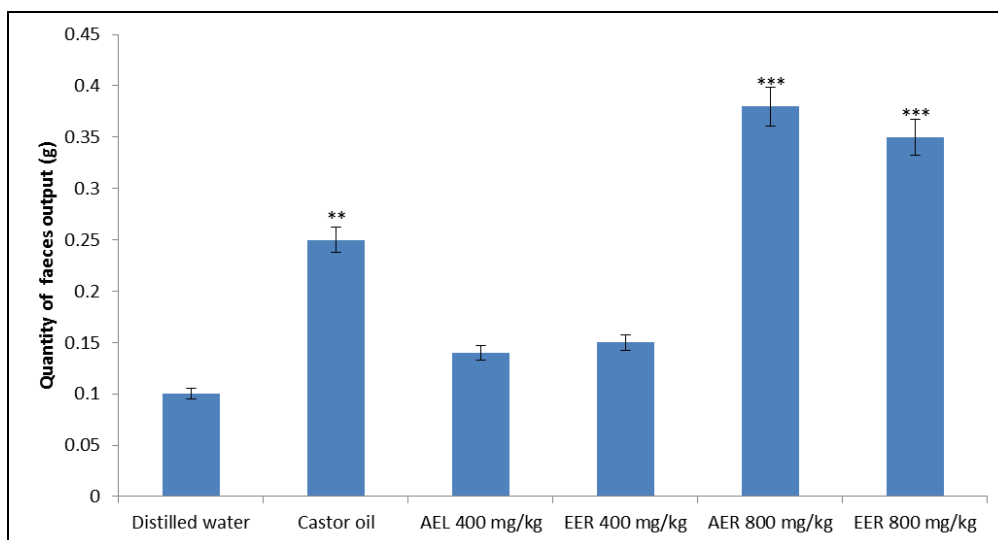


Fig 6: Effect of *Anchomanes difformis* leaves and rhizome on total quantity of faeces output on loperamide induced constipation rats. Values given are mean \pm S.E.M of five observations, *** p < 0.001 and ** p < 0.01 compared with the control group. AER : aqueous extract of rhizome, AEL : aqueous extract of leaves, EER: ethanolic extract of rhizome

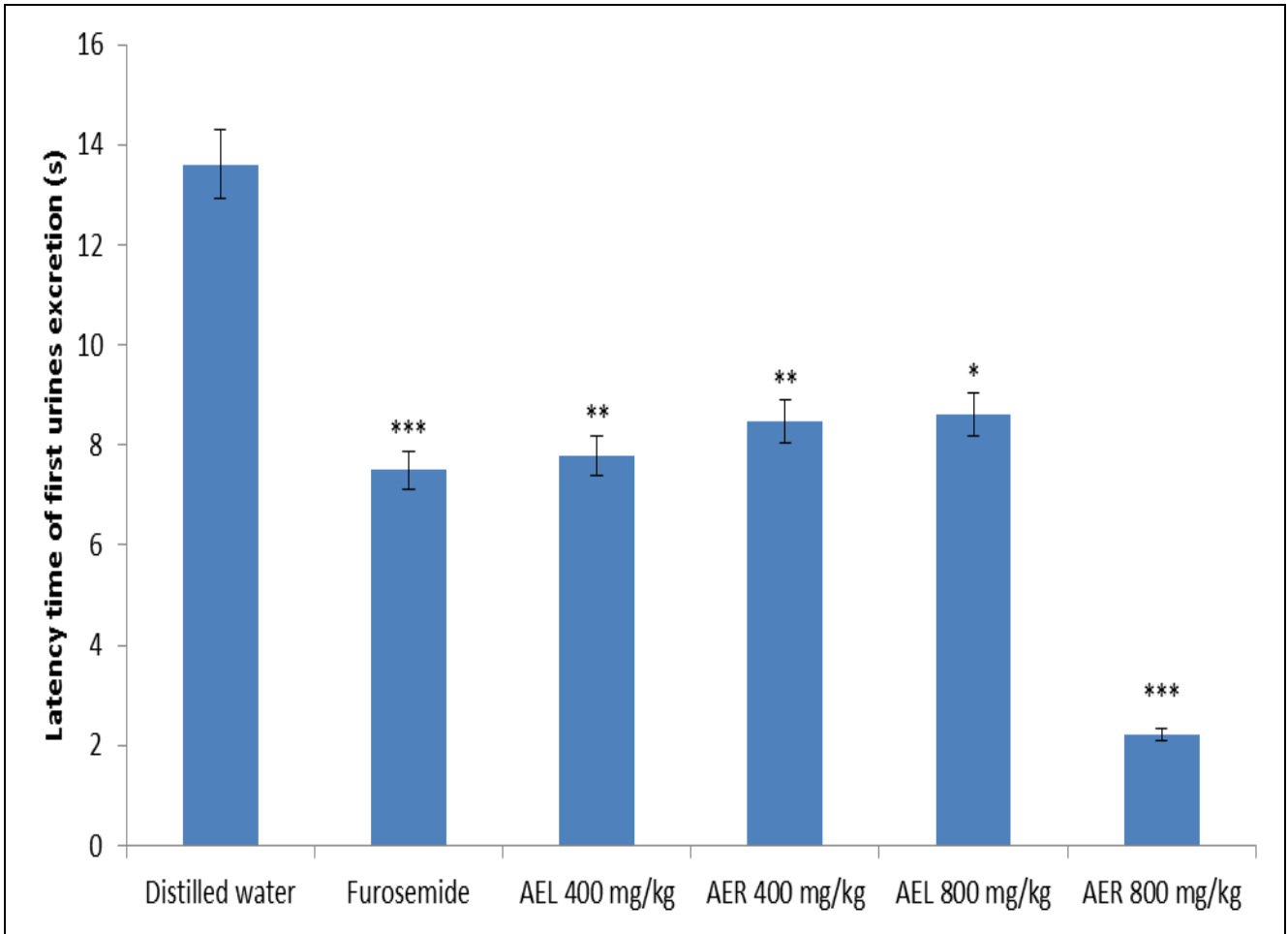


Fig 7: Effect of *Anchomanes difformis* leaves and rhizome aqueous extracts on latency time of first urines excretions in rats. Values shown are mean \pm S.E.M (n = 5) and the values are compared with control group and considered significant at *** $p < 0.001$; ** $p < 0.01$ and * $p < 0.05$. AER : aqueous extract of rhizome, AEL : aqueous extract of leaves

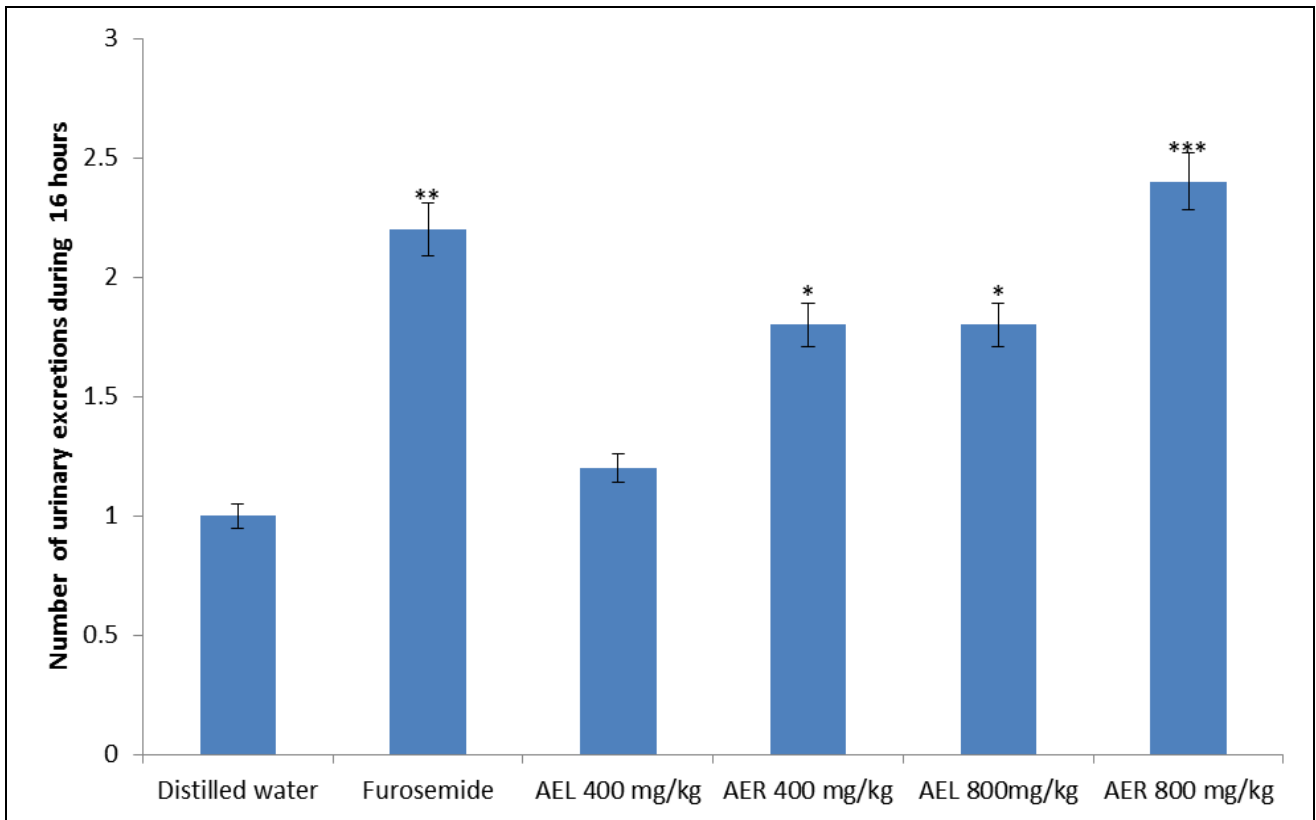


Fig 8: Effect of *Anchomanes difformis* leaves and rhizome aqueous extracts on frequency of urinary excretions in rats. Values shown are mean \pm S.E.M (n = 5) and the values are compared with control group and considered significant at *** $p < 0.001$; ** $p < 0.01$ and * $p < 0.05$. AER : aqueous extract of rhizome, AEL : aqueous extract of leaves

4. Discussion

In this research, the phytochemical screening, laxative and diuretic the effects of leaves and rhizome of *Anchomanes difformis* were investigated. An Increase in latency time of first feces excretion, of frequency of defecation and the quantity of feces are Among the Most significant characteristics of laxative (Gangarosa *et al.*, 2003) [8].

The classic phytochemical tests are well known and report the chemical composition of plant extracts (Abe and Lajide, 2014) [2]. The result of phytochemical analysis is not opposed to those found by our predecessors (Tchiakpe *et al.*, 1980; Bekro *et al.*, 2012; Lajide and Abe, 2014; and Idu Ataman, 2015) [24, 14, 2].

Numerous authors report the results of studies of the laxative effect expressed by the total mass of fecal material excreted from 6 to 8h and at 16h after treatment. In this study we have modified this protocol considering only the total amount of fecal matter excreted 16 hours after treatment, which does not alter the effect obtained, and adding two parameters; latency time of first fecal matter excretion and frequency of fecal excretion during the observation period. In fact, the chemical composition of the extracts and the doses used may interfere with the mass of excreted fecal matter in the extent that a portion of the extract will be the matter. As it happens exactly with the original vegetable foods ingested by mammals. To do this we firstly evaluated the time to onset of raw fecal matter that accounts for the rate of absorption of the active ingredients and the speed of action, and secondly, we also evaluated the frequency excretory materials which sometimes reflects the laxative effect whatever the physical condition of the stool, and sometimes justified, indirectly, the existence of large stools massively. And we do not forget that some laxatives do not act quickly and help eliminate a significant amount of fecal matter without several times of fecal excretion. We also analyzed the physical quality of the stool because we thought that it could afford to speculate on the likely mechanism of action of a laxative product and establish a relationship between the quality of the stools and urine output. In fact, diarrhoea and soft stools contain plenty of water and evokes a direct water retention mechanism by osmosis by passing a flow of water from the blood plasma to the intestinal lumen, thus limiting urinary water excretion (Kakino Mamoru *et al.*, 2012) [13], this is the case of osmotic laxatives such as sugars (Washabau, 2003) [28]. Soft stools may also justify a lubricating effect of some laxative such as paraffin oil (Gangarosa and Seibert 2003) [8]. While hard stools evoke a stimulating effect on intestinal motility or increased fecal mass associated with the presence of fiber or mucilages. Foul odors aware of intense activity of intestinal microflora (Gangarosa and Seibert 2003) [8]. The laxative effect was confirmed in rats constipated by loperamide. Indeed, loperamide acts directly on the nervous system and causes intestinal constipation (Mamoru Kakino *et al.*, 2012) [13]. Only the most effective doses in the study of the laxative activity in normal rats were selected to be evaluated on the loperamide-induced constipation. The aqueous and ethanolic extracts of the rhizomes have a strong laxative effect the more important than that observed with castor oil used as a reference, which is noticed by the total quantity, excretion rate and the time of first latency excretion of feces at the dose of 800 mg/kg. And this activity is very strong and remarkable in normal or constipated rats. The aqueous extract of the leaves showed a significant laxative activity on the total amount of

feces at the doses of 400 and 800 mg/kg in normal rats. But this effect disappears completely in the constipated rats. This disappearance may explain a need to increase the dose. The effect of extracts on first fecal excretions latency can be attributed to rapid absorption of the active ingredients at the intestinal wall. On the physical characteristics, stool test groups are morphologically identical to the stool of the control group. It is therefore not a direct or indirect effect of the extracts on the physical characteristics of stool. This allows excluding the hypothesis of laxatif mechanism by osmolarity or induction of a hydroelectric disorder. Therefore, *Anchomnes difformis* laxatif mechanism of action passes through a stimulation of intestinal motility, like it has been demonstrated by Bekro *et al.* (2012) [14]. This effect would be the numbers by the presence of metabolites that were found in these extracts. Indeed, anthraquinons, alkaloids, saponins, tannins and terpenes have already demonstrated a laxative effect (Abah *et al.*, 2011, Eke Ifeanyi *et al.*, 2013; Oyetayo, 2007) [1, 5].

Liptschiz method was modified by adding two additional measures; the time of first urine excretion and the frequency of urinary excretions are two parameters that contribute to the expression of a diuretic (Umang Patel *et al.*, 2009) [26] and which account implicitly on speed intestinal absorption and the approximate duration of the half-life. Drug is considered as good diuretic when the diuretic index is greater than 1,5 and the value of Liptschiz > 0,5 (Gasparotto *et al.*, 2009) [9]. The diuretic index between 1 and 1.5 indicates a moderate diuretic and Liptschiz values between 0,72 and 0,99 a weak effect. For latency urinary excretions, the effect of aqueous extracts of the rhizomes (400 mg/kg) and leaves (400 and 800 mg/kg) is significant and comparable to furosemide used as reference. While the effect of the extract of the rhizome at 800 mg/kg is far superior to furosemide. This shows that these extracts are rapidly absorbed and reach effective plasma concentration and half-life very quickly. As regards the frequency of urinary excretions all extracts have a significant effect. Unlike that only rhizome extract at 800 mg/kg have slightly higher activity as furosemide, other treatments were less effective than furosemide. The total volume of urine excreted in rats treated at 800 mg/kg showed a significant difference from the controls, but this effect is substantially the same as furosemide (** $p < 0.01$) with 1.74 of diuretic index for the aqueous extract of the leaves (** $p < 0.01$) and 2,35 for the extract of the rhizome (***) $p < 0.001$). The diuretic index of furosemide was 1.94. Concerning Lipschitz value at the up dose of 800 mg/kg, the rhizome extract showed 120% of diuretic activity and the leaves extract showed 90% as Compared with furosemide. This effect is due to the presence of alkaloids, flavonoids, steroids, terpenoids and glycosides present both in the leaves and rhizomes (Umang *al.* Patelet, 2009) [26]. This study complements the work already begun by Bekro *et al.* (2012) [14], as regards the study of the laxative effect, which have demonstrated for the first time the existence of *Anchomanes difformis* stimulatory effect on the guinea pig ileum *in vitro*, and that raised the possibility of a laxative effect. In this study laxatif effect have demonstrated in an animal model of a mammalian (rat) *in vivo* after oral administration of *Anchomanes difformis* extracts using safeties doses. This work have showed also diuretic effect *in vivo* after oral administration of the extracts for first time. Both effects explain the use of this plant against constipation, anuria, edema and hypertension in traditional medicine.

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