The Journal of Phytopharmacology 2016; 5(5): 182-184
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2016; 5(5): 182-184
September- October

Alain N'guessan Yao
Department of Clinical Pharmacology, UFR-SMA, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire

Mamadou Kamagaté
Department of Clinical Pharmacology, UFR- Medical Sciences Bouaké, Alassane Ouattara University, Bouaké, Côte d'Ivoire

Augustin Kouao Amonkan
Laboratory of Nutrition and Pharmacology, UFR-Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire

Camille Koffi
Department of Clinical Pharmacology, UFR-SMA, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire

Fidèle Kpahe
Laboratory of Nutrition and Pharmacology, UFR-Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire

Mathieu N'Goran Kouamé
Department of Clinical Pharmacology, UFR-SMA, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire

Henri Dié-Kacou
Department of Clinical Pharmacology, UFR-SMA, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire

Correspondence:
Alain N'guessan Yao
Department of Clinical Pharmacology, UFR-SMA, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire
22 BP 582 Abidjan 22, Abidjan, Côte d'Ivoire
Email: yalain08[at]yahoo.fr
Tel: +225 77120473; Fax: +225 445795

Comparative effects of aqueuos extract of *Phyllanthus amarus* and its fractions on urinary excretion in rat

Alain N'guessan Yao*, Mamadou Kamagaté, Augustin Kouao Amonkan, Camille Koffi, Fidèle Kpahe, Mathieu N'Goran Kouamé, Henri Dié-Kacou

ABSTRACT

The aim of the present study was to compare the effect of *Phyllanthus amarus* extracts and its fractions on urinary excretion. Aqueous extract of *Phyllanthus amarus* was prepared by decoction of the whole plant and lyophilized. Ethanolic fraction and chloroformic fraction of *Phyllanthus amarus* were obtained from aqueous extract. Animal were divided into 5 groups of 6 rats and placed individually in metabolic cages. The control group received normal water. A positive control group received furosemide (5 mg/kg, i.p.), used as a reference loop diuretic drug. Three other groups were treated with aqueous extract or ethanolic fraction or chloroformic fraction of *Phyllanthus amarus* by intraperitoneal injection at the same dose of 40 mg/kg. Urine volumes were collected each 2 h during 8 h period. The diuretic action was obtained by a ratio of urinary excretion of treated group and that of control group. *Phyllanthus amarus* extracts increased urinary excretion. This effect was time dependant and significant, compared to control group ($p < 0.001$). During all experiments, ethanolic fraction increased urinary excretion, more than other extracts. After 8h period, it had eliminated about 2.44 ± 0.27 mL, however this value remained less than that of furosemide (3.01 ± 0.17 mL). The urinary excretion induced by furosemide was significantly high ($p < 0.05$), compared to ethanolic fraction, but the ratio was similar. This study showed that, like furosemide, ethanolic fraction seemed to be the most potent extract for diuresis. Further studies might be carried out to identify the actives molecules and its mechanisms.

Keywords: *Phyllanthus amarus*, Urinary excretion, Furosemide, Euphorbiaceae.

INTRODUCTION

Diuretic drugs are important in the treatment of several cardiovascular and renal diseases such as hypertension, heart failure, eclampsia, nephritis and chronic renal failure [1, 2]. They contribute to the regulation of blood pressure, promoting significant urinary excretion of water and electrolytes (sodium, potassium, chlorure), decreasing blood volume and peripheral resistance [3]. Their renoprotective effects also result in removal of metabolite of protein and toxic substances [4]. In order to potentiate the therapeutic effect, diuretics can be used as combination therapy with others standard antihypertensive drugs. The pharmaceutical use of outpatient diuretics is limited, because of their potential side effects such as electrolyte imbalances, metabolic alterations, activation of the renin-angiotensin system (RAS) and neuroendocrine and sexual function disturbances [2, 5]. In this context, the research of biomolecules as diuretic agent, is an alternative and a public health key challenge [6]. Several plant extracts such as *Hibiscus sabdariffa*, and *Allium sativum*, are used in traditional medicine against high blood pressure for their diuretic power [7]. Also, *Phyllanthus amarus* could have diuretic activity [8]. Previous work has shown that aqueous extract of *Phyllanthus amarus* administered intraperitoneally (i.p.) in normotensive rabbits at doses ranging from 4.5 to 71.7 mg/kg, decreased transiently mean arterial pressure. The ethanol fraction seemed more active than the other extracts tested [9]. The presence of diuretic component in *Phyllanthus amarus* extract may contribute to potentiate it hypotensive effect. The aim of the present study was to compare the effect of *Phyllanthus amarus* and its fractions on urinary excretion.

MATERIAL AND METHODS

Extraction and fractionation

The whole plant of *Phyllanthus amarus* (Euphorbiaceae) was collected in the district of Cocody (Abidjan, Côte d'Ivoire) in April 2014. This plant was authenticated by a botanist at the *Centre National de Floristique* (CNF, UFR Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire). The samples are kept for this species (herbarium No. 3, 141 and 248). Aqueous extract of *P. amarus* and its fractions were prepared according to a method previously described, with a minor modification [2]. Whole plants were harvested and washed brought to boiling in distilled water for 5 to10 min at 500 g for 1 liter. The decoction was filtered and then lyophilized to obtain a powder of aqueous extract of *P. amarus* (EAPA). Three grams of lyophilisate were dissolved in 250 mL of 70 % ethanol solution using a

separating funnel for 12 hours. The ethanol phase was collected and dried using a rotary evaporator (Buchi). The powder obtained represented the ethanolic fraction of *P. amarus* (EFPA). In next step, 5 g of AEPA was dissolved in 300 mL of water and suspended in a separating funnel of 1000 mL. From this solution, two extraction were done using cyclohexane (3 × 600 mL) and chloroform (3 × 600 mL). This operation allowed to obtain after evaporation the chloroformic fraction of *Phyllanthus amarus* (CFPA). After preparation, extracts were stored at 5 °C, before using.

Animal

Male Wistar rats (180 -250 g) were acclimatized for 14 days in the animal house of the *Ecole Normale Supérieure (Abidjan)* under standard conditions (25 ± 2 °C) with dark and light cycle (12/12 h) and had free access to a standard dry pellet diet and water *ad libitum*. Experimental procedures and protocols were approved by Ethical Committee of Health Sciences of Félix Houphouët-Boigny University, (Abidjan, Côte d'Ivoire).

Diuretic activity

Animals were divided into 5 groups of 6 rats and placed in metabolic cages (one in each cage). The group I received normal water and served as control group. The group II received Furosemide (5 mg/kg, i.p.) from Tocris Bioscience (Abingdon, UK), used as a reference loop diuretic drug. The group III, IV and V were treated respectively with AEPA, EFPA and CFPA by intraperitoneal injection at the same dose of 40 mg/kg. Fluid overloading was done *per os* at 50 mL/kg and animal received immediately extracts or drug test. Urines were collected every 2 h for 8 h periods. The ratio of the volume of urine excreted and the volume of fluid overloaded was used to determine urinary volumetric excretion (UVE) according to method described in previous study [10]. The diuretic action was obtained by a ratio of urinary excretion of treated group and of control group [11].

Statistical Analysis

The statistical analysis was performed using Graph Pad Prism 5 software (Microsoft, San Diego, California, USA). Differences between the groups were assessed using one-way analysis of variance (ANOVA) of the multiple test of comparison of Tukey-Kramer. $p < 0.05$ was considered significant. All values are expressed as mean ± SEM.

RESULTS

The present study showed that aqueous extract of *Phyllanthus amarus* and its fractions (ethanolic and chloroformic) at dose of 40 mg/kg increased urinary output in rat. These effects were time dependant and significant, compare to control group ($p < 0.001$). After 2 h, the urinary output induced by EAPA, CFPA and EFPA were respectively 0.53 ± 0.1 mL, 0.70 ± 0.1 mL and 0.86 ± 0.08 mL (Fig. 1). The urinary volumetric excretion corresponding were respectively 29.45 ± 1.08 %, 41.2 ± 2.77 % and 42.60 ± 1.70 % (Fig. 2). In the same period, volume of urine eliminated by furosemide, a reference loop diuretic drug was estimated to 0.91 ± 0.12 mL (urinary volumetric excretion : 45.52 ± 1.05 %). During all experiments, EFPA increased urinary excretion, more than other extracts (Fig. 2). After 8h period, EFPA at 40 mg/kg eliminated about 2.44 ± 0.27 mL, however this value remained significantly lesser than that of furosemide (3.01 ± 0.17 mL; Fig.1). From 6 h to the end of experiment, the urinary excretion induced by furosemide was significant ($p < 0.05$), compared to *Phyllanthus amarus* extracts (Fig. 2). The diuretic action of furosemide (5 mg/kg, i.p.), and EFPA at 40 mg/kg seemed more important than that of CFPA and AEPA over 8 h period ($p < 0.05$; Table 1). This ratio was not significantly different between furosemide and EFPA.

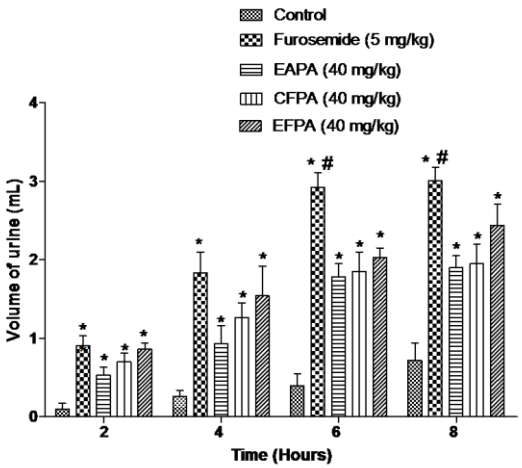

Figure 1: Comparative effets of *Phyllanthus amarus* extracts (40 mg/kg) and furosemide (5 mg/kg) on elimination of water overload in rats over 8 h period. Each value represents the mean ± SEM of six rats.* $p < 0.001$versus control group and $^{\#}p < 0.05$, furosemide versus EFPA group.

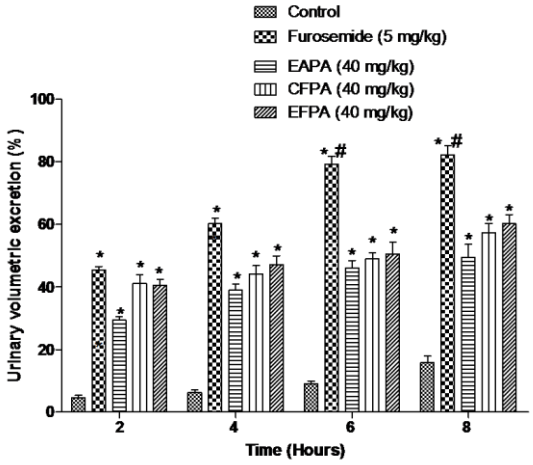

Figure 2 : Comparative effets of *Phyllanthus amarus* extracts (40 mg/kg) and furosemide (5 mg/kg) on urinary volumetric excretion in rats after 8 h period. Each value represents the mean ± SEM of six rats.* $p < 0.001$versus control group and $^{\#}$ $p < 0.05$, furosemide versus EFPA group.

Table 1 : Diuretic action of *Phyllanthus amarus* (40 mg/ kg) extract and furosemide (5 mg/kg) in rats after 8 h period.

Treatment	Doses (mg/kg)	Diuretic action
Control	0	1
Furosemide	5	4.18*
EFPA	40	3.33*
CFPA	40	2.70*
AEPA	40	2.63*

* $p < 0.05$ versus control group.

DISCUSSION

This study evaluating the diuretic effect of three extracts showed that EFPA was the most powerfull. This diuretic action of EFPA at 40 mg/kg was greater than that of alcoholic extract of *Pergularia daemia*, at 400 mg/kg, confirming the potent effect of EFPA needed in ethnomedicine as diuretic or antihypertensive agent [11].

Using urinary excretion is a good method for evaluating the biological activity of plants used in the treatment of hypertension or kidney desease [12]. Also, the diuretic effect of *Phyllanthus amarus* extracts varied according to the polarity of solvents used. EFPA exhibited urinary excretion more than other extracts. Similar results were reported for methanol fraction of *Whithania aristata* which induced

interesting diuretic activity compared to other fractions of the plant [13]. These findings releaved that, although the substances responsible of the diuretic effect are unknow, the actives compounds were preferably extracted in organic solvents with high polarity such as ethanol. However, previous phytochemical studies have indicate that *Phyllanthus amarus* contain several secondary metabolites such as alcaloïdes, flavonoïdes, polyphenols, saponins, terpenes and sterols [14, 9]. These compounds may interact individually or synergically on kidney receptor to increase urine output [15]. Interesting, early study showed that polyphenolic compounds, flavonoïdes, saponins and triterpenoids have diuretic effect [16-18]. This will be more salient to identify these compounds to elucidate the main mechanism underlying. Generally, diuresis was associated with significant excretion of electrolytes. So, the interaction of *Phyllanthus amarus* with electrolytes such as sodium, potassium or chlorure could permit to sign up the diuretic effect and predict the way of action on electrolytes balance. In the light of the diuretic effect, EFPA seemed less active than furosemide according to urinary excretion or volumetric exctretion, but exhibited a similar diuretic action timely dependant according to urinary excretion ratio (Table 1). This activity could be used in case of emergency, for hypertension crisis reactions or acute renal failure like furosemide. Finally, this effect contribute to justify its antihypertensive property.

CONCLUSION

Altogether, our study show that, like furosemide, *Phyllanthus amarus* extract and its fractions exhibited in dose-dependent manner an significant rise in the urine output in rats. Ethanolic fraction seemed to be the most potent extract for diuresis. Further study might elucidate it mechanism and the main active metabolite.

Acknowledgments

Authors are thankful to the authorities of *Ecole Normale Supérieure (Abidjan)* for providing support to the work. This study was partially supported by a scholarship from *Ministère de l'Enseignement Supérieur et de la Recherche Scientifique de Côte d'Ivoire (Décision N° 436/MERS/DBE/SD-BHCI du 11/09/2013).*

Conflits of Interests

No conflicts of interest.

REFERENCES

1. Gupta S, Neyses L. Diuretic usage in heart failure: a continuing conundrum in 2005: Eur Heart J 2005; 26 : 644-49.
2. Wile D. Diuretics: a review. Ann Clin Biochem 2012; 49: 419-31.
3. Ntchapda F, Kakesse M, Fokam MA, Pancha OM, Abakar D, Dimo T. Evaluation of the diuretic effects of crude stem bark extraction of *Zanthoxylum heitzii* (Rutaceae) in Wistar rats. J Integr Med 2015;13: 326-35.
4. Kurkin VA, Zaitseva EN, Kurkina AV, Dubishchev AV, Pravdivtseva OE. Bull Exp Biol Med 2015; 159: 368-71.
5. Ellison DH, Loffing J. Thiazide effects and adverse effects: insights from molecular genetics. Hypertension 2009 ; 54 :196-202.
6. Ribeiro EF, de Fátima Reis C, de Carvalho FS, Abreu JP, Arruda AF, Garrote CF, Rocha ML. Diuretic effects and urinary electrolyte excretion induced by *Aspidosperma subincanum* Mart. and the involvement of prostaglandins in such effects. J Ethnopharmacol 2015; 163:142-8.
7. Wright CI, Van-Buren L, Kroner CI, Koning MM. Herbal medicines as diuretics: A review of the scientific evidence. J Ethnopharmacol 2007;114: 1-31.
8. Srividya N, Periwal S. Diuretic, hypotensive and hypoglycemia effect of *Phyllanthus amarus*. Indian J Exp Biol 1995; 33: 861- 4.
9. Amonkan AK, Kamagaté M, Yao ANR, Konan AB, Kouamé MN, Koffi C, Kati-Coulibaly S, Die-Kakou H. Comparative effects of two fractions of *Phyllanthus amarus* (Euphorbiaceae) on the blood pressure in rabbit. Greener J Med Sci 2013a; 3: 129-34.
10. Amonkan AK, Konan AB, Ahui, BML, Bleyéré MN, Kouakou LK, Bouaffou GMK. Diuretic effects of extract of *Ficus exasperata* Vahl. leaves in rat. Pak J Biol Sci 2013b; 16:1383-7.
11. Bhavin V, Ruchi V, Santani DD. Diuretic potential of whole plant extracts of *Pergularia daemia* (Forsk.). Iran J Pharm Res 2011; 10 : 795-8.
12. Diallo D, Guissou IP, Haïdara M, Tall C, Kasilo OMJ. Recherche sur la médecine traditionnelle africaine: Hypertension. Afri Health Monit 2010;4 : 58 -63.
13. Martín-Herrera D, Abdala S, Benjumea D, Gutiérrez-Luis J. Diuretic activity of some *Withania aristata* Ait. fractions. J Ethnopharmacol 2008; 117 : 496-9.
14. N'guessan K, Beugré K, Zirihi GN, Traoré D, Aké AL. Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte d'Ivoire). Sci Nat 2009; 6: 1-15.
15. Ahmed MM, Andleeb S, Saqib F, Hussain M, Khatun MN, Ch BA, Rahman H. Diuretic and serum electrolyte regulation potential of aqueous methanolic extract of *Solanum surattense* fruit validates its folkloric use in dysuria. BMC Complement Altern Med 2016; 16: 166.
16. Jadhav RB, Bhatnagar SP, Surana SJ. Diuretic activity of squamate mistletoe, *Viscum angulatum*. Pharm Biol 2010; 48: 417- 21.
17. Jiménez-Ferrer E, Alarcón-Alonso J, Aguilar-Rojas A, Zamilpa A, Jiménez-Ferrer CI, Tortoriello J, Herrera-Ruiz M. Diuretic effect of compounds from *Hibiscus sabdariffa* by modulation of the aldosterone activity. Planta Med 2012; 78: 1893-8.
18. Toma C, Olah N, Vlase L, Mogoşan C and Mocan A. Comparative studies on polyphenolic composition, antioxidant and diuretic effects of Nigella sativa L. (black cumin) and *Nigella damascena* L. (Lady-in-a-Mist) seeds. Molecules 2015; 20: 9560-74.

HOW TO CITE THIS ARTICLE

Yao NG, Kamagaté M, Amonkan AK, Koffi C, Kpahe F, Kouamé MN, Dié-Kacou H. Comparative effects of aqueuos extract of *Phyllanthus amarus* and its fractions on urinary excretion in rat. J Phytopharmacol 2016;5(5):182-184.