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Antihypertensive effect of *Dialium guineense* Wild. and *Trema orientalis* L. in L-NAME-induced hypertensive rats

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ABSTRACT

Dialium guineense (Caesalpiniaceae) and *Trema orientalis* (Celtidaceae) are traditionally used to treat and manage many diseases such as hypertension, headache and diabetes. Despite their recognized efficacy in traditional medicine, their antihypertensive properties are not yet effective. Therefore, the current study aimed to investigate the antihypertensive effect of ethanolic extracts of *Dialium guineense* and *Trema orientalis* on L-NAME-induced hypertensive rats. The systolic, diastolic and mean arterial pressure were recorded using CODA™ non-invasive blood pressure system. The phenolic compounds were also quantified using High Pressure Liquid Chromatography. Ethanolic extracts of both plants induced significant decrease of mean arterial pressure. At 500 mg/kg bw, both plants decreased mean arterial pressure from 126.4 ± 0.48 to 90.6 ± 3.12 mmHg and from 154.8 ± 7.84 to 103 ± 5.6 mmHg respectively for *Dialium guineense* and *Trema orientalis*. Losartan and Captopril, used as standard drugs at 100 mg/kg body weight, also significantly decrease blood pressure. *Trema orientalis* was the most active with a blood pressure reduction percentage of 33.46 ± 3.06 % comparable to Losartan (31.37 ± 3.13 %) and Captopril (31.76 ± 2.63 %). The probable bioactives compound identified were chlorogenic, gallic, caffeic, ellagic, tannic acids, and luteolin, isorhamnetin and chrysin.

Keywords: *Dialium guineense*, *Trema orientalis*, Antihypertensive activity.

INTRODUCTION

All over the world, human health is affected by the same problems, such as the aging of the population, rapid urbanization and the spread of unhealthy lifestyles. People in developed and low income countries face the same health problems, among which the most important is the increase of cases of non transmissible diseases such as cardiovascular diseases, cancer, diabetes and chronic lung diseases [1]. Although World Health Organization announced that governments ensure that all people have equitable access to the preventive, curative and rehabilitative health services they need to avoid developing high blood pressure and its complications, it is clear that the road ahead to achieve this goal is still long [1]. In spite of the increase of accessibility of modern medicine and the variety of drugs available for various ailments, it has been observed that 85% of patients combine herbal therapy with the medicines prescribed at hospitals or clinics [2]. The plant-derived remedies or recipes are widely used in all civilizations and cultures. Hence, plants have always played an important role in health care systems worldwide [3]. Despite the availability of various approaches and strategy for the discovery of new drugs, natural products still remain as one of the best reservoirs of new structural types of compounds. Indeed, It is well known that plants are a valuable source of new natural and pharmaceutical products. It has also been reported that plants and plant-based products constitute the bases of many modern pharmaceuticals used today for the treatment of various ailments [4]. In Bénin, medicinal plants are used in the management of various diseases including hypertension (HTA) [5, 6]. These plants include *Dialium guineense* Wild., and *Trema orientalis* L. belonging to the family Caesalpiniaceae and Celtidaceae respectively [7]. *Dialium guineense* is widely used in folk medicine to manage various ailments [8, 9] whereas *Trema orientalis* has been reported for the management of some diseases such as hypertension, malaria and diarrhea [10, 11]. Several biological properties such as antiplasmodial, antioxidant, analgesic, anti-microbial were also carried out on *Trema orientalis* [12, 13]. Despite their frequent use and studies, as far as we know, there is no report on the antihypertensive activity of *Dialium guineense* and little data are available about the antihypertensive activity of *Trema orientalis*. Consequently, the effect of chronic administration of ethanolic extracts of *Dialium guineense* and *Trema orientalis* on L-NAME-Induced Hypertensive Wistar rats was investigated and the probable bioactives phenolic secondary metabolites were quantified.

MATERIALS AND METHODS

Plant materials

Fresh sample of *Dialium guineense* Wild. (Caesalpinaceae) and *Trema orientalis* Linn. (Celtidaceae) were harvested in South of Bénin, department of Atlantic in November 2015. The collected specimen was identified and validated by taxonomists from National Herbarium of Université d'Abomey-Calavi where the registration numbers (YH 284/HNB; YH 262/HNB) were attributed. The specimens were deposited at the same herbarium.

Preparation of extract

The stem bark of *Dialium guineense* and leafy stem of *Trema orientalis* were washed briefly with distilled water and dried in laboratory under air-conditioned ($22^{\circ}\text{C} \pm 2$). Then, Dried plant material were grinded to fine particles by an electric grinder (MARLEX Electroline Excella). Three hundred grams (300 g) of powdered plants were macerated with 1 L of ethanol for 24 hours. The obtained mixture was filtered using a Whatman No.1 paper filter. The extraction process was repeated twice on the residue and all filtrate was concentrated under reduce pression using rotary evaporator (BUCHI Rotavapor RII). The obtained extracts (*D. guineense*: 28.02 g and *T. orientalis*: 34.94 g) were stored at 4°C for assay.

Quantitative analysis of phenolic compounds in extracts

Quantitative analysis of phenolics compounds in *Dialium guineense* and *Trema orientalis* ethanolic extracts was performed using U-HPLC 3000 liquid chromatography system, equipped with a DAD - 3000 RS and MWD- 3000 RS UV detector and a reversed phase column (C_{18} , 150×4.6 mm, $5 \mu\text{m}$ Hypersil BDS). The mobile phases consisted of water (A) and acetonitrile (B) with 0.1% formic acid each. The elution gradient and sample characteristics were presented in Table 1. Chromleon v.6.80 Software (Dionex, Thermo Fisher Scientific) was used to analysis data. The probable bioactive metabolites were identified by comparison with standard compounds (retention times, UV-Vis spectra) and data from literature.

In-vivo arterial pressure measurement

Experimental animals

Male Wistar albino rats weighing 200 to 250 g were used for the experiment. All animals were maintained under constant laboratory conditions ($23 \pm 2^{\circ}\text{C}$), 12 h day/night cycle and free access to a standard diet and water. They were subjected to experimental conditions for two weeks in order to accustom them to the experimental equipment and minimize stress during evaluation of antihypertensive activity of extracts. Blood pressure was measured by the tail-cuff technique using a Non-Invasive System ("CODA™ 20942" Kent Scientific Corporation). The experiments were carried out following the guidelines for the use of laboratory animals of the Faculty of Health Science and Faculty of Sciences and Technologies of University of Abomey-Calavi.

Hypertension induction and treatment

N(G)-Nitro-L-Arginine-Methyl Ester (L-NAME) was orally administered to the rats to induce the hypertension. After two weeks of L-NAME administration, the hypertensive animals were treated with the reference drugs (Losartan and captopril) and the ethanolic extracts of *Dialium guineense* and *Trema orientalis* (Table 2). For the experiment, a total of forty (40) rats were used. They were divided into eight (08) groups of five (5) animals each. The distilled water was used to dissolve reference drugs and extracts (L-name, Losartan, Captopril and crude extract).

Antihypertensive effect evaluation

During the experiment, Systolic (SAP), Diastolic (DAP) and Mean arterial pressure (MAP) were measured to evaluate the antihypertensive efficacy of the extracts and also the heart rate (HR). All the parameters were measured once weekly for four (04) weeks using the CODA™ non-invasive Blood Pressure system. The method is based on volumetric blood flow/blood volume to measure systolic and diastolic blood pressure in the tail [14]. Animals were placed in their holder on a heat platform to improve blood flow to the tail and reduce movements during measurements. For each experiment, blood pressure was measured twenty times, composed of five (5) initial measurements for equipment stabilization and fifteen (15) experimental measurements. Among these, at least seven measure selected and recorded by the equipment as valid data were used for analysis. The percentage of reduction of SAP, DAP and MAP was calculated using the formula below:

$$\% \text{ Red} = \frac{\text{SAP (d15)} - \text{SAP (d29)}}{\text{SAP (d15)}} \times 100$$

%Red: Percentage of blood pressure reduction; **SAP (d15):** Mean SAP values at day 15; **SAP (d29):** Mean SAP values at day; the same formula was used for DAP and MAP; (n = 5).

Ethical consideration

The experimental protocol was approved under registration number UAC/FAST/ED-SVT/10132309 by scientific committee of Doctoral School of Life and Earth Sciences of University of Abomey-Calavi.

Statistical analysis

All the obtained results were expressed as the mean \pm standard deviation (SD). The antihypertensive results were analyzed using STATA version 14.0 software. Linear regression was used to evaluate the degree of significance (*P values* < 0.05) of the induction of hypertension and the effect of extracts.

RESULTS AND DISCUSSION

Quantitative analysis of phenolics compound

The phenolic compounds identified in each extract by U-HPLC-DAD were resumed Table 3. The analysis of *Dialium guineense* extract revealed the presence of three phenolic acid (gallic, chlorogenic, tannic acid) and two flavonoids (Luteolin, Isorhamnetin) whereas *Trema orientalis* extract revealed chlorogenic, tannic, ellargic acids, luteolin, isorhamnetin and chrysin. Four and eight non-identified compounds were also detected respectively in *Dialium guineense* and *Trema orientalis* (Fig. 1). These identified phenolic compounds are known for their numerous pharmacological potentials and their beneficial effects for the management of various pathologies. Indeed, many studies reported their interesting effects as cardioprotective, antioxidant, anticancer, hypolipidemic, antihypertensive and anti-inflammatory [15-20].

Effet of L-NAME on arterial pressure and heart rate

During the four weeks of experimentation, no significant changes in MAP were observed in the control group (105.2 ± 7.44 to 101 ± 4.4 mmHg). Chronic administrations of L-NAME at 40 mg/kg body weight daily caused a significant increase of SAP, DAP and MAP in animals when compare to control group (Figure 2, 3). It is know that L-NAME is commonly used to induce hypertension in experimental animals. The increase of blood pressure during chronic L-NAME administration is linked with NO deficiency. Thus, inhibition of nitric oxide (NO) synthesis after L-NAME administration could explain the increase of arterial blood pressure mainly via its oxidative stress effect [21]. The NO level reduction causes its imbalance with ROS, thus leading to endothelial dysfunction and blood pressure elevation [22, 23]. It is well

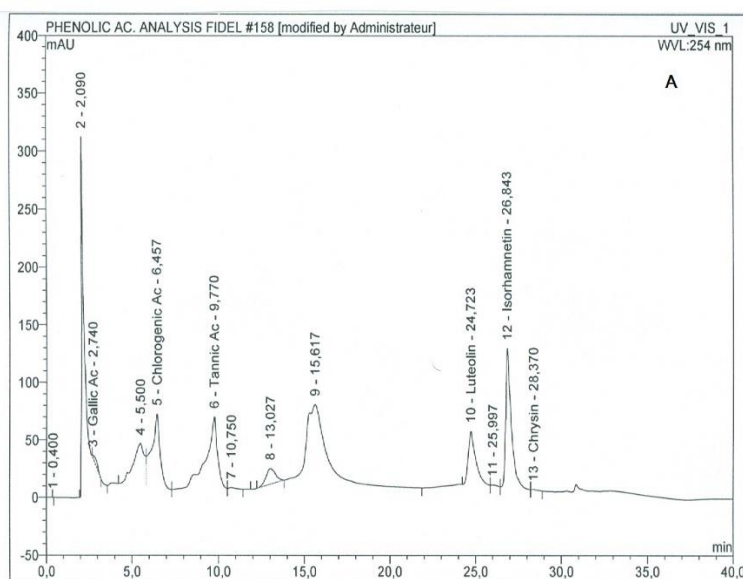
documented that chronic blockade of NO synthesis by NOS inhibitors like L-NAME lead to endothelial dysfunction, significant increase in arterial blood pressure and further pathological injuries to the cardiovascular system and kidneys, which may lead to aggravation of hypertension condition [24]. Then, the significant increase in SAP, DAP and MAP after L-NAME administration was well justify in the present study. Contrary to arterial pressure, in same conditions, significant decrease in the heart rate was observed when compared to control. These results could be explained by the fact that the NO produced by eNOS could play a direct role in the modulation of the heart rate [14]. Inhibition of NO production after L-NAME administration could lead to a decrease in heart rate. However, the data obtained in our study do not allow to rule on the real effect of the reduction of heart rate on animals.

Effect of *Dialium guineense* and *Trema orientalis* on blood pressure and heart rate

The use of medicinal plants as a source of first line medicines for primary health care and the management of several ailment remains a reality. This practice has become universally popular, especially in developing countries, where plants are considered as a source of health security. Several surveys on the use of medicinal plants [25, 26] and mainly regarding the management of hypertension [7, 27] confirmed this assertion. The two studied species, *Trema orientalis* and *Dialium guineense*, are used to manage hypertension in southern Bénin and remains of great therapeutic interest which still unexplored or little explored. Thus, we investigated the ability of ethanolic extracts of stem bark of *Dialium guineense* and leafy stem of *Trema orientalis* to reduce blood pressure.

Significant decrease of the SAP, DAP and MAP of all experimental groups was observed after administration of ethanolic extracts of *Dialium guineense* and *Trema orientalis* at 250 and 500 mg/kg body weight (bw) for two weeks following L-NAME administration. From day 15 to 29, at 250 mg/kg bw and 500 mg/kg bw, *Dialium guineense* extract decreased MAP respectively from 139 ± 6.4 to 111.4 ± 7.52 mmHg and from 126.4 ± 0.48 to 90.6 ± 3.12 mmHg (Fig. 2). As far as we know, this is the first time that the antihypertensive activity of *Dialium guineense* was reported. In the same conditions, *Trema orientalis* extract decreased MAP from 146 ± 4.8 mmHg to 98.4 ± 14.08 mmHg and at 500 mg/kg bw, MAP was decreased from 154.8 ± 7.84 to 103 ± 5.6 mmHg (Fig. 3). The standard drug, losartan and captopril, also reduced MAP from 122.2 ± 06.16 to 83.00 ± 4.80 mmHg and 127.4 ± 03.52 to 87.20 ± 3.36 mmHg respectively. In general, both ethanolic extracts of *Dialium guineense* and *Trema orientalis* induced a dose

dependent significant decrease of SAP, DAP and MAP. However, *Trema orientalis* extract was the most active with a blood pressure reduction percentage of 33.46 ± 3.06 % comparable to standard drugs, Losartan (31.37 ± 3.13 %) and Captopril (31.76 ± 2.63 %) (Table 4). The decrease in blood pressure observed after administration of the extracts may be associated with the restoration of endothelial system functions through the restoration of NO synthesis that had been blocked by L-NAME administration. Our previously study revealed the presence of various phytoconstituents such flavonoids, tannins, alkaloids, lignanes, triterpenes, cardiac glycosides, coumarin, saponin, essential oils, anthracene derivatives. Considering that the therapeutic effect of a plant is generally the result of the combination of secondary metabolites, the decrease in blood pressure induced by the ethanolic extracts could be attributed to the secondary metabolites identified during the previous phytochemical characterization. In addition, the antihypertensive effect of plants is attributed to their ability to scavenge ROS, as the oxidative stress is considered as a risk factor for some non-communicable diseases such as hypertension and cardiovascular diseases [28]. In the present study, the analysis of *Dialium guineense* and *Trema orientalis* ethanolic extracts using high pressure liquid chromatography allowed identify phenolic acids and flavonoids which could contributed to their antihypertensive activity. The cardioprotective properties of flavonoids and phenolic compounds have been proved by their various mechanisms including inhibition of ROS generation, mitochondrial dysfunction, apoptosis [29, 23]. Previous studies have shown that regular consumption of phenolic compounds such as flavonoids or foods with high contain in flavonoid can significantly improve oxidative status as well as endothelial function [30-32]. Thus, the decrease of MAP could be associated with the phenolic and flavonoids content of the extracts. The antioxidant activity of both extracts has been also described in previous studies. It is well documented that some Antioxidant agents produced an antihypertensive effect through endothelium-independent vasodilation action and vascular protective effect under oxidative stress conditions [33, 23]. Besides to flavonoids and phenolic acids, most of the secondary metabolites detected in the plants extracts are well known for their ability to decrease high blood pressure [34, 35]. It is also reported that an increased consumption of foods rich in polyphenols content such as fruits and vegetables reduced the risk of developing of cardiovascular diseases and then cardiovascular death [23]. Administration of L-NAME to rats significantly decreased the heart rate. Ethanolic extracts of *Dialium guineense*, *Trema orientalis* and reference drugs (Losartan and Captopril) reestablished ($P < 0.05$) the heart rate (Table 5). These results could be due to the restoration of NO production that had been blocked by L-NAME administration.



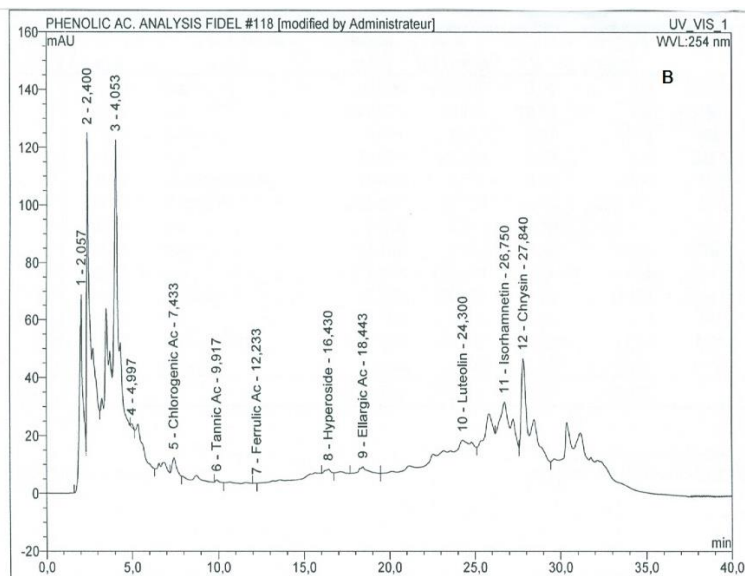


Figure 1: Chromatograms of quantitative analysis of phenolic compounds of *Dialium guineense* (A) and *Trema orientalis* (B) ethanolic extracts

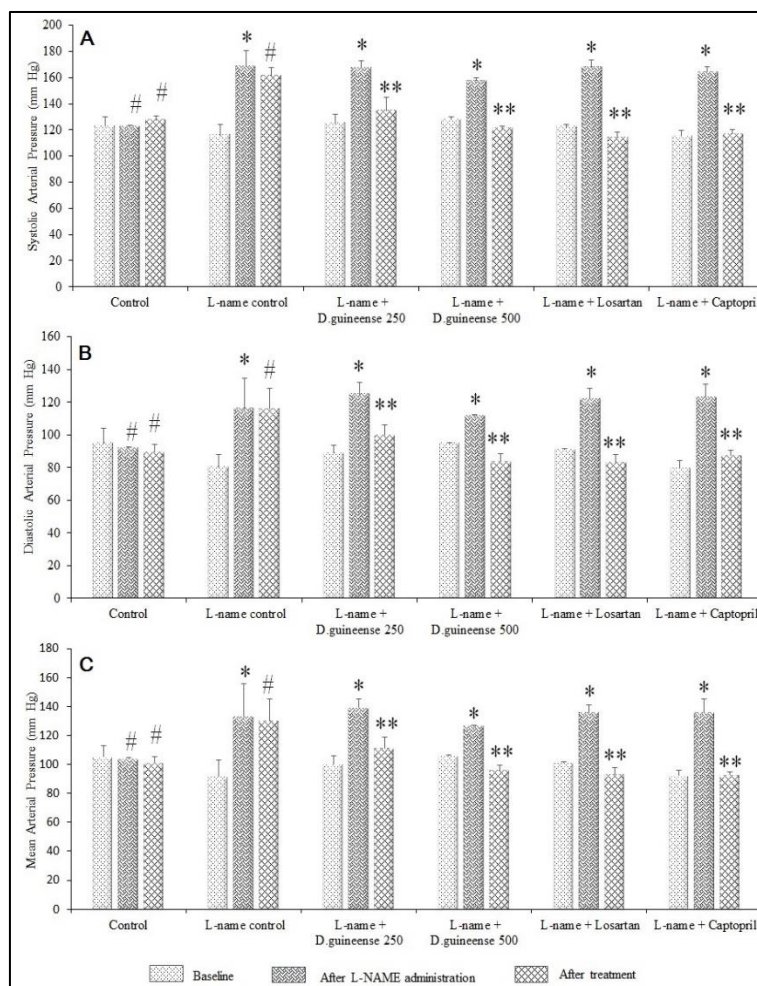


Figure 2: Effect of ethanolic extract of *Dialium guineense* on Systolic (A); Diastolic (B) and Mean Arterial Pressure (C) in L-NAME-Induced Hypertensive Wistar rats

Control: Systolic; Diastolic and Mean Arterial Pressure of rats that received only water during the experimental period ; **L-name control:** Systolic; Diastolic and Mean Arterial Pressure of rats that received only N(G)-Nitro-L-Arginine-Methyl Ester for hypertension induction during two weeks and observation during the following two weeks; **L-name + *D. guineense* 250 and 500 :** rats treated with extracts at 250 and 500 mg/Kg body weight for two weeks after hypertension induction. * ($p < 0.05$): significant change after hypertension induction ; ** ($p < 0.05$): significant change after hypertension treatment ; # ($p > 0.05$): no significant change of SAP; DAP and MAP.

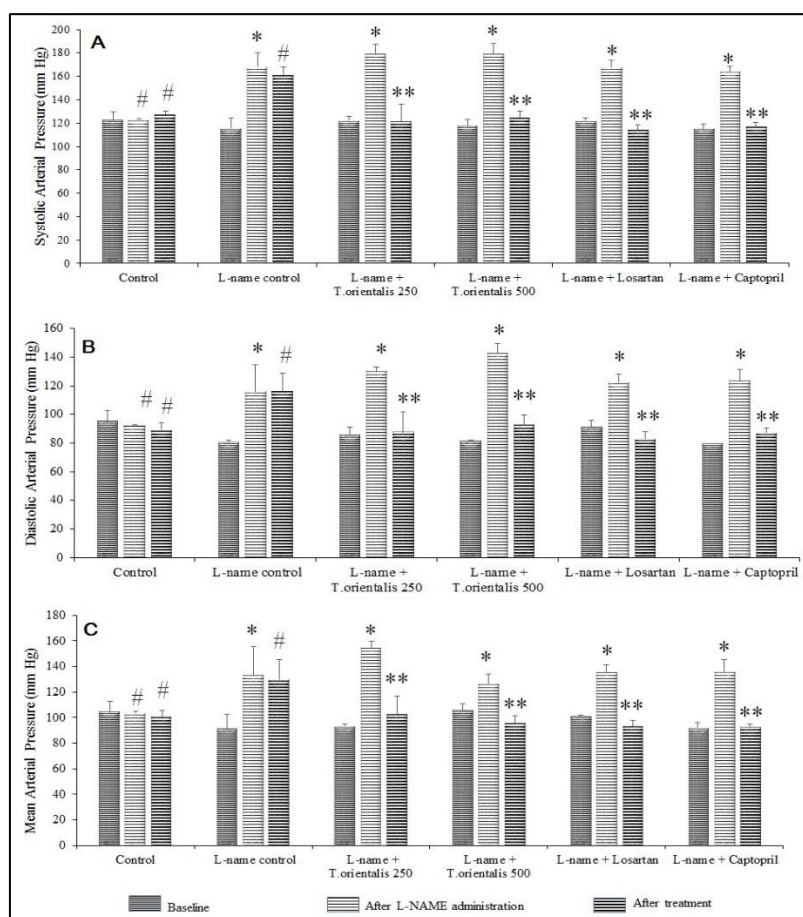


Figure 3: Effect of ethanolic extract of *Trema orientalis* on Systolic (A); Diastolic (B) and Mean Arterial Pressure (C) in L-NAME-Induced Hypertensive Wistar rats

Control: Systolic; Diastolic and Mean Arterial Pressure of rats that received only water during the experimental period ; **L-name control:** Systolic; Diastolic and Mean Arterial Pressure of rats that received only N(G)-Nitro-L-Arginine-Methyl Ester for hypertension induction during two weeks and observation during the following two weeks; **L-name + *T. orientalis* 250 and 500 :** rats treated with extracts at 250 and 500 mg/Kg body weight for two weeks after hypertension induction. * ($p < 0.05$): significant change after hypertension induction ; ** ($p < 0.05$): significant change after hypertension treatment ; # ($p > 0.05$): no significant change of SAP; DAP and MAP.

Table 1: Elution gradient during quantitative analysis of phenolic compounds

Time (min)	A (H ₂ O, 1% formic acid)	B (ACN, 1% formic acid)
00	80	20
20	50	50
25	30	70
30	20	80
35	80	20
40	80	20

H₂O : distilled water ; ACN : acetonitrile

Table 2: Hypertension induction and treatment

Animals Groups	Distilled water	L-NAME (40 mg/kg bw)	Ethanolic Extract (mg/Kg bw)				Standards (mg/kg bw)	
			<i>Dialium guineense</i>		<i>Trema orientalis</i>		Losartan	Captopril
			250	500	250	500	100	100
Group 1	+	-	-	-	-	-	-	-
Group 2	-	+	-	-	-	-	-	-
Group 3	-	+	-	-	+	-	-	-
Group 4	-	+	-	-	-	+	-	-
Group 5	-	+	+	-	-	-	-	-
Group 6	-	+	-	+	-	-	-	-
Group 7	-	+	-	-	-	-	+	-
Group 8	-	+	-	-	-	-	-	+

(+) : administrated ; (-) not administrated ; Five (n=5) animals per group

Table 3: Phenolic compounds identified in *Dialium guineense* and *Trema orientalis* ethanolic extracts by U-HPLC 3000

Standards	Retention time (min)	Amount ($\mu\text{g/g}$ of extract)	
		<i>D. guineense</i>	<i>T. orientalis</i>
Gallic acid	2.74	0.032	nd
Chlorogenic acid	6.95	2.096	0.094
Caffeic acid	7.47	nd	nd
Tannic acid	9.85	358.373	1.308
Ferrulic acid	--	nd	nd
Rutin	--	nd	nd
Ellagic acid	18.44	nd	0.011
Luteolin	24.51	0.304	0.056
Isorhamnetin	26.80	6.820	1.207
Chrysin	28.11	0.002	0.188
Hyperoside	--	nd	nd
Gallic acid	--	nd	nd
Syringic acid	--	nd	nd
Quercetin	--	nd	nd

nd: not detected

Table 4: Percentage (%) of blood pressure reduction after administration of *Dialium guineense* and *Trema orientalis* ethanolic extracts

Treatment	[C] (mg/kg.bw)	Percentage of blood pressure reduction (%)		
		SAP	DAP	MAP
<i>Dialium guineense</i>	250	19.57 \pm 4.93	20.16 \pm 6.52	19.86 \pm 5.76
	500	23.18 \pm 2.19	25.09 \pm 4.29	28.32 \pm 2.43
<i>Trema orientalis</i>	250	32.07 \pm 08.85	32.77 \pm 11.38	32.60 \pm 10.31
	500	30.44 \pm 4.60	35.11 \pm 3.43	33.46 \pm 3.06
Losartan	100	32.14 \pm 3.69	32.08 \pm 3.79	31.37 \pm 3.13
Captopril	100	28.87 \pm 3.70	31.55 \pm 3.96	31.76 \pm 2.63

SAP : Systolic arterial pressure ; DAP : Diastolic arterial pressure ; MAP : Mean arterial pressure

Table 5: Effect of ethanolic extract of *Dialium guineense* and *Trema orientalis* on Heart rate in L-NAME-Induced Hypertensive Wistar rats

Treatment	[C] (mg/kg.bw)	Baseline (Beginning)	After L-NAME administration	After treatment
Control	--	277.80 \pm 32.24	267.80 \pm 36.24 [#]	307.40 \pm 36.24 [#]
L-NAME	40	266.00 \pm 34.40	189.40 \pm 42.64*	304.80 \pm 15.84*
<i>Dialium guineense</i>	250	350.20 \pm 20.16	277.60 \pm 57.12*	340.60 \pm 51.68*
	500	262.00 \pm 22.40	146.60 \pm 07.12*	356.60 \pm 02.88*
<i>Trema orientalis</i>	250	330.8 \pm 28.24	202.4 \pm 10.48*	302.8 \pm 44.32*
	500	322.00 \pm 26.4	208.2 \pm 25.04*	269.4 \pm 36.88*
Losartan	100	294.00 \pm 36.80	205.00 \pm 25.60	264.00 \pm 22.00*
Captopril	100	308.00 \pm 28.00	247.00 \pm 30.40*	310.60 \pm 02.72*

Control : rats that received only water during the experimental period ; L-name : rats that received only N(G)-Nitro-L-Arginine-Methyl Ester for hypertension induction during two weeks and observation during the following two weeks; *($p < 0.05$): significant change of heart rate ; # no significant change.

CONCLUSION

In conclusion, these obtained results suggest that the ethanolic extract of *Dialium guineense* and *Trema orientalis* at 250 and 500 mg/kg bw exhibited antihypertensive effect by lowered arterial pressure. The phenolic acids and flavonoids identified in both extracts contributed to the biological activities observed. However, further studies is ongoing in other to determine the mechanism of action of the extract to reduce arterial pressure. Apart from antihypertensive mechanism of the extracts, various studies will be performed to consider these plants be safety as part of the management of high blood pressure.

CONFLICTS OF INTEREST

The authors declare there is no conflicts of interest.

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