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UHPLC-DAD characterization of bioactive secondary metabolites from *Ocimum americanum* and *Pupalia lappacea* extracts: Antioxidant activity and antihypertensive effects on L-NAME-induced hypertensive rats

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Ocimum americanum L. (Lamiaceae) and *Pupalia lappacea* (L.) Juss. (Amaranthaceae) are two plants used in Bénin to manage hypertension. Little scientific data is available on the antihypertensive properties of these plants. Therefore, we investigated the antihypertensive potential of ethanolic extracts of *O. americanum* and *P. lappacea* on L-NAME-induced hypertensive rats. The DPPH free radical scavenging potential, Fe³⁺ reducing capacity, superoxide anion radical and hydrogen peroxide scavenging were assessed. Extracts were also screened for their active compounds using ultimate high-performance liquid chromatography 3000. CODA™ non-invasive blood pressure system was used to record blood pressure parameters. Both extracts induced significant decrease of systolic, diastolic blood pressure and mean arterial pressure. *O. americanum* extract at 250 mg/kg body weight, decreased mean blood pressure (MBP) from 146 ± 4.80 to 98.4 ± 9.44 mmHg and *P. lappacea* extract from 154.4 ± 11.28 to 111.8 ± 9.44 mmHg. A significant decrease of MBP was also observed with Losartan and Captopril at 100 mg/kg body weight. *P. lappacea* showed the highest ferric reducing/antioxidant power 4905 ± 87.79 μmol AAE g⁻¹. Superoxide anion and hydrogen peroxide scavenging activities showed higher activity, 68.42 ± 3.68 and 38.68 ± 4.18%, respectively for *O. americanum*. The chromatography analysis of extracts suggested the presence of ferulic acid, chlorogenic, tannic, ellagic, caffeic acids and chrysin, rutin and isorhamnetin. The obtained results justify the traditional use of *O. americanum* and *P. lappacea* in management of hypertension in southern Bénin.

Key words: *Ocimum americanum*, *Pupalia lappacea*, antihypertensive activity, antioxidant activity.

INTRODUCTION

Hypertension is reported next to many infectious diseases as most serious health problems in developing tropical countries (Orch et al., 2015). The reasons for

increasing prevalence of hypertension can be correlated to exposure to persistent stress, excessive alcohol consumption, use of tobacco unhealthy diet, physical

consumption, use of tobacco unhealthy diet, physical inertness, excess weight and ageing (WHO, 2013). Oxidative stress is also one of the reasons for the occurrence of hypertension. Reactive oxygen species (ROS) are associated with many vascular risk factors, including hypertension (Amoussa et al., 2015). Thus, the control of hypertension becomes imperative given the high mortality and morbidity associated with its complications (WHO, 2009). According to the World Health Organization, more than 80% of people in developing countries still depend on local medicinal plants to fulfill their primary health needs (WHO, 2009). In Bénin, medicinal plants are used in the treatment of various pathology among which the arterial hypertension (HTA). Over the last three decades, many collaborative efforts have been devoted to research on local plants with hypotensive and antihypertensive effects (Raji et al., 2013). Today, research on therapeutic potential of medicinal plants has become a global issue, as medicinal plants are the richest source of medicines in traditional medicine systems, modern medicines, nutraceuticals, dietary supplements, traditional medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Pandey and Tripathi, 2014). Also, the importance of plants in medicine remains even of greater relevance with the current global shift to obtain drugs from plants sources, as a result of which, attention has been given to the medicinal value of herbal remedies for safety, efficacy and economy (Tsobou et al., 2015). In the field of hypertension, several plants are used in traditional medicine. *Ocimum americanum* is widely used alone or in combination to treat many diseases such as hypertension, skin disease, dysentery, digestive, stomachic, genitorurinary, lowering blood glucose and also treats cold, fever, parasitic infestations, inflammation of joints and headaches (Karou et al., 2011; Lagnika et al., 2016). Antibacterial, antimalarial, antioxidant and insecticidal activities have been also reported (Ntonga et al., 2014). *Pupalia lappacea* is reported in folk medicine for various purposes. It is used to treat urethra pain, leprosy, fractured bone, endometritis, cystitis and leucorrhoea and as laxative, purgative, anti vomitory, antisterility, anti emetic and antalgic (Naidu et al., 2014; Srinivas, 2015). Many studies have demonstrated its anticonceptive, hepatoprotective, antipyretic, anti-inflammatory properties and antimicrobial, antidiarrhoeal activity (Mehnoor and Chakrapani, 2015; Naidu et al., 2014; Apenteng et al., 2014; Hoekou et al., 2012). Despite their traditional use, there is little or no information to confirm the antihypertensive properties attributed empirically to these plants. Therefore, this study is designed to investigate the effects of chronic administration of ethanolic extracts of *O. americanum* (OAE) and *P. lappacea* (PLE) to L-NAME-

induced hypertensive Wistar rats, to evaluate the antioxidant potential and quantify phenolic compounds of each extract.

MATERIALS AND METHODS

Plant materials

Fresh samples of *O. americanum* and *P. lappacea* were collected in Southern Bénin in July 2015. The plants were identified and authenticated at the National Herbarium of the University of Abomey-Calavi where the voucher specimens were deposited; *O. americanum* (YH 277/HNB) and *P. lappacea* (YH 234/HNB).

Preparation of extracts

Selected plants were dried in laboratory under air-conditioned ($22 \pm 2^\circ\text{C}$) and then reduced to powder using an electric grinder (MARLEX Electroline Excella). 300 g of each plant were extracted with 1 L of ethanol under stirring for 24 h. The macerate was filtered through a Whatman No.1 paper filter and concentrated using a rotary evaporator (BUCHI Rotavapor RII). The extraction process was repeated three times. The obtained extracts were stored at 4°C for assay.

Phytochemical investigation

Thin layer chromatography (TLC) and colorimetric methods tandem with high pressure liquid chromatography analysis were used.

Qualitative phytochemical assay

Flavonoids, tannins, alkaloids, triterpenes, coumarins, saponins, essential oils, lignans, pigments, naphthoquinones, anthracene derivatives and cardiac glycosides were characterized according to standard methods using TLC (Wagner and Bladt, 2001) and colorimetric reaction (Shah and Hossain, 2010).

Total phenolic contents

Total phenolics of extracts were determined according to methods used previously (Amoussa et al., 2015). Total phenolic content was measured spectrophotometrically using Folin–Ciocalteu reagent. The absorption of sample was read at 765 nm against a blank and gallic acid was used as the standard. The total phenolic was calculated using the equation of the calibration curve of gallic acid and expressed as gallic acid equivalent (mg GAE g^{-1} dry weight).

Total flavonoid content

The total flavonoid of extracts was determined by a colorimetric assay using aluminium chloride, and the absorbance was read at 415 nm (Amoussa et al., 2015). Quercetin was used as reference. Total flavonoid was expressed as quercetin (mg/g) using the equation of the calibration curve of quercetin. Total flavonoids are

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reported as milligrams of quercetin equivalent (QE) per 100 g of extract.

Identification and quantification of active compounds by U-HPLC 3000

Preparation of samples

Stock solutions at 100 µg/ml in methanol of thirteen standards phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid, tannic acid, ellagic acid, gallic acid, syringic acid, luteolin, chrysin, rutin, hyperoside, quercétol and and isorhamnetin) were prepared and stored at 4°C. Appropriate dilutions were performed prior to analysis. The ethanolic extract of each plant was also prepared at 1 mg/ml in methanol. All samples and standards solutions were filtered with 0.2 µm pore sizes filters.

U-HPLC 3000 analysis

The standard phenolic compounds analysis and quantification were carried out using U-HPLC 3000 liquid chromatograph system, equipped with a degasser, binary gradient pump, a UV multiwavelength detector (DAD - 3000 RS and MWD - 3000 RS) and a C₁₈ reversed phase column (150 × 4.6 mm, 5 µm Hypersil BDS) at ambient temperature. The mobile phases consisted of water (A) with 0.1% formic acid and acetonitrile (B) with 0.1% formic acid. The elution gradient (0-20 min, 20-50% B; 20-25 min 50-70% B; 25-30 min, 70-80% B; 30-35 min, 80-20% B; 35-40 min, 20% B). The flow rate was 1 ml/min and injection volume 20 µl. Data analysis was performed using Chromleon v.6.80 Software (Dionex, Thermo Fisher Scientific). Phenolic compounds in extracts were identified according to their retention times, UV-Vis spectra and comparison with standard compounds.

Antioxidant activity

Antioxidant activity of OAE and PLE was evaluated using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) scavenging, ferric-reducing antioxidant power (FRAP) assay, superoxide anion scavenging activity and hydrogen peroxide scavenging assay.

DPPH radical-scavenging activity

The free radical scavenging capacity of extracts was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described by Talbi et al. (2015). The extracts were solubilized in dimethyl Sulfoxide (DMSO) and then diluted to obtain a stock solution at 100 µg/ml which is subjected to two-fold dilutions to make eight concentrations. The test consists of 1.5 ml of the freshly prepared 2% DPPH methanolic solution and 0.75 ml of each extract. Methanolic solutions of DPPH and ascorbic acid were used as blank and reference, respectively. After 15 min incubation in darkness, absorbance was measured at 517 nm using spectrophotometer (VWR UV-1600 PC). All experiments were performed in triplicate. The inhibition power of the DPPH radical, expressed as a percentage, is calculated according to the formula below:

$$\text{Inhibition (\%)} = [(AB - As) / AB] \times 100 \quad (1)$$

Where: As; tested extract absorbance and AB; blank absorbance.

Ferric-reducing antioxidant power (FRAP) assay

The ability to reduce ferric ions was measured using the method of

Saeed et al. (2012). The test mix consists of 2 ml of extracts (100 µg/ml) in ethanol, 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferricyanide (10 mg/ml). The mixture was incubated at 50°C for 20 min followed by addition of 2 ml of trichloroacetic acid (100 mg/ml). The obtained solution was centrifuged at 3000 rpm for 10 min. 2 ml of supernatant were mixed with 2 ml of distilled water and 0.4 ml of 0.1% fresh ferric chloride (w/v). After 10 min incubation, the absorbance was read at 700 nm. Ascorbic acid was used to produce the calibration curve. Assay was performed in triplicate and expressed in µMol Ascorbic Acid Equivalent (AAE)/g of extract.

Superoxide anion scavenging activity

A modified version of the method described by Kumar was used (Kumar et al., 2012). The superoxide radicals were generated by alkaline DMSO. All extracts and nitro blue tetrazolium (Sigma, N6639) were prepared in DMSO at 100 µg/ml and 1 mg/ml respectively. The test mixture consist of 50 µl of extract was mixed with 170 µl of alkaline DMSO and 30 µl of nitro blue tetrazolium (NBT). After 5 min incubation at laboratory temperature (22°C ± 2), the absorbance was measured at 630 nm against blank using microplate reader (Rayto R 6500, China). The blank consist of the reaction mixture without extract. Quercetin was used as standard. All the experiments were performed in triplicate. Superoxide anion scavenging percentage (SP) was calculated as follow:

$$\text{SSP} = [(AB - As) / AB] \times 100 \quad (2)$$

Where: SSP; Superoxide scavenging percentage, As; extract absorbance and AB; blank absorbance.

Hydrogen peroxide scavenging assay

Hydrogen peroxide (H₂O₂) scavenging potential was determined using the method of Mohan et al. (2012). A solution of hydrogen peroxide (100 mM) was prepared in phosphate buffer (0.1 mM, pH 7.4). The reaction mixture consists of 0.5 ml of extract at 100 µg/ml diluted in distilled water and 1.5 ml of hydrogen peroxide solution at 40 mM. After 10 min, the absorbance was measured at 295 nm using spectrophotometer (VWR UV-1600 PC). The phosphate buffer solution and gallic acid were used as blank and standard respectively. Assay was performed in triplicate. The H₂O₂ radical scavenging was calculated as bellow:

$$\text{HSP} = [(AB - As) / AB] \times 100 \quad (3)$$

Where: HSP; Hydrogen peroxide scavenging percentage, As; extract absorbance and AB; blank absorbance.

Blood pressure measurement

Animal

Male Wistar rats weighing 200 to 250 g obtained from the Laboratory of Human Biology, Faculty of Health Sciences, University of Abomey-Calavi were used. The selected animals were maintained under laboratory conditions (24 ± 2°C), exposed to 12 h day/night cycle, and free access to a diet and water. They were subjected to experimental conditions for two weeks in order to accustom them to blood pressure measure equipment and then minimizing stress during the experiment. Blood pressure was measured by the tail-cuff method using a non-invasive blood

pressure system for rats (Kent Scientific CODA™ 20942). The study was done in accordance with the guidelines for the care and 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

Hypertension was induced in rats by administration of N(G)-Nitro-L-Arginine-Methyl Ester (L-NAME). After confirmation of the hypertensive status, animals were treated with the ethanolic extracts and the reference drugs; losartan and captopril. Forty (40) rats divided into eight (8) groups of five (5) animals are used. The first group received distilled water from day 1 to 28. Groups 2 to 8 received L-NAME at 40 mg/kg body weight from day 1 to 14. These groups were subsequently treated with reference drugs and ethanolic extracts from day 14 to 28. Group 2 received distilled water. Groups 3 and 4 received respectively, losartan and captopril at 100 mg/kg body weight. Groups 5 and 6 respectively received OAE at 250 and 500 mg/kg body weight, whereas Groups 7 and 8 received respectively, PLE at 250 and 500 mg/kg body weight. All substances and extracts (L-name, losartan, captopril and crude extracts) were prepared in distilled water and administrated orally to rats.

Antihypertensive evaluation

At the end of the treatment, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP) and heart rate (HR) were measured to assess the antihypertensive effect of extracts. All parameters are measured on days 1, 8, 15, 22 and 29 using the CODA™ non-invasive blood pressure system (Kent Scientific Corporation) which is based on blood flow to measure systolic and diastolic blood pressure in the tail of animal (Sung et al., 2013). Animals were placed in their sockets on a heated platform to improve blood flow to the tail and minimize movements when taking measurements. During each experiment, blood pressure was measured 20 times including five (5) initial acclimation measurements and 15 experimental measurements. Among the experimental measurements, at least seven (7) were considered valid by the CODA™ system. Valid measurements were used for statistical analysis.

Ethical consideration

The experimental protocols used in this study were approved by the scientific committee of the Doctoral School of Life Sciences and Earth at University of Abomey-Calavi (UAC/FAST/EDSV/ 10132309).

Statistical analysis

All the results obtained are presented as mean \pm standard deviation form. Results of the antihypertensive activity were analyzed using STATA version 14.0 software. Linear regression was used to evaluate the degree of significance of the induction of arterial hypertension and the effect of extracts. The level of significance was set at 0.05.

RESULTS

Phytochemical analysis

A similarity was noticed within secondary metabolites of

both plants. Flavonoids, triterpenes, coumarins, lignanes, anthocyanines and essential oils are the secondary metabolites identified in both extracts. Contrary to *P. lappacea*, tannin, alkaloids, naphthoquinones and anthracene derivatives were also detected in *O. americanum*.

Total phenolic and flavonoids contents

The total phenolic of *O. americanum* (28.15 ± 0.23 mg EAG/100 mg) is higher than *P. lappacea* (13.77 ± 1.12 mg EAG/100), whereas total flavonoids are comparable in both extracts with 48.06 ± 0.82 mg EQ/100 mg for *O. americanum* and 49.90 ± 1.85 mg EQ/100 mg for *P. lappacea*.

Identification and quantification of actives compounds using U-HPLC 3000

Analysis of ethanolic extracts allowed to identified ferulic acid, caffeic acid, chlorogenic acid, tannic acid, ellargic acid, chrysin and rutin in OAE while ferulic acid, chlorogenic acid, tannic acid, ellargic acid, chrysin and rutin were identified in PLE. The results of the quantitative analysis by HPLC were presented in Table 1. Other unidentified molecules also appeared in the extracts.

DPPH radical-scavenging activity

The antioxidant activity of OAE and PLE are dose-dependent as described in Figure 1. At 100 μ g/ml, DPPH scavenging activity of *O. americanum* ($43.50 \pm 2.12\%$) is higher than that of *P. lappacea* ($25.52 \pm 1.54\%$). Compared to ascorbic acid ($99.46 \pm 0.38\%$) used as the control, the plant extracts had moderate activity.

Ferric-reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) assay is based on the reduction of ferricyanide complex (Fe^{3+}) to ferrous form (Fe^{2+}) by antioxidant metabolite in extracts. Ethanolic extract of *P. lappacea* showed the highest ferric reducing power (4905 ± 87.79 μ mol AAE g^{-1}) compared to *O. americanum* (4745.9 ± 113.39 μ mol AAE g^{-1}) as shown in Table 2.

Superoxide anion and hydrogen peroxide scavenging activities

The superoxide anion and hydrogen peroxide scavenging activities of *O. americanum* and *P. lappacea* extracts are shown in Table 2. In superoxide anion assay, *O.*

Table 1. Actives phenolic compounds identified in *O. americanum* and *P. lappacea* extracts by U-HPLC 3000.

Standard	Retention time (min)	Amount (mg/g)	
		<i>O. americanum</i>	<i>P. lappacea</i>
Chlorogenic acid	7.19	0.089	0.051
Caffeic acid	7.47	0.031	nd
Tannic acid	10.11	6.349	0.001
Ferrulic acid	12.21	0.043	0.031
Rutin	17.87	0.505	0.001
Ellargic acid	18.63	0.200	0.040
Isorhamnetin	27.07	0.549	nd
Luteolin	nd	nd	nd
Chrysin	28.05	0.478	0.011
Hyperoside	nd	nd	nd
Gallic acid	nd	nd	nd
Syringic acid	nd	nd	nd
Quercetin	nd	nd	nd

nd: not detected.

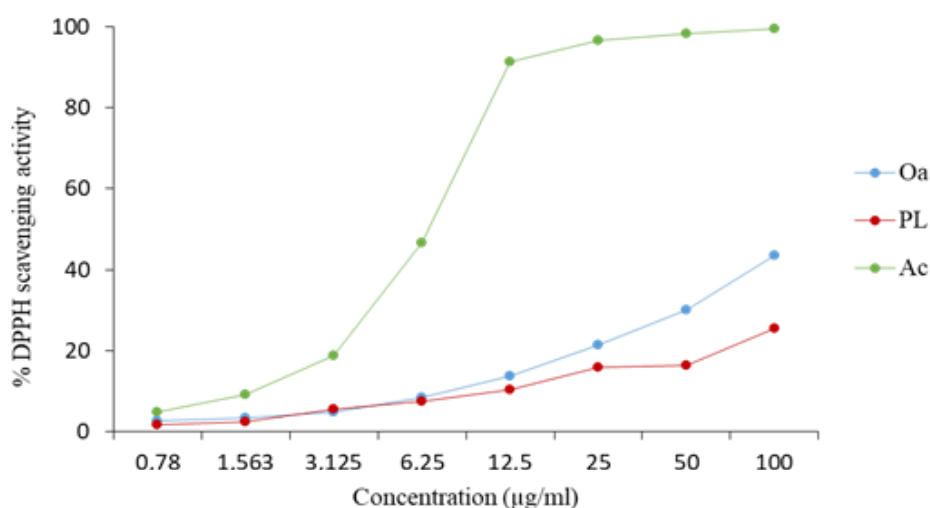


Figure 1. Radical DPPH scavenging activities of *O. americanum* and *P. lappacea* extracts. Oa: *O. americanum*; PL: *P. lappacea*; Ac: Ascorbic acid.

Table 2. Superoxide anion, hydrogen peroxide scavenging and ferric reducing antioxidant activities of *O. americanum* and *P. lappacea*

Extract	Superoxide anion (%)	Hydrogen peroxide (%)	Ferric reducing antioxidant potential ($\mu\text{mol AAE g}^{-1}$)
<i>O. americanum</i>	68.42 \pm 3.68	38.68 \pm 4.18	4905 \pm 87.79
<i>P. lappacea</i>	58.76 \pm 3.42	32.67 \pm 2.45	4745.9 \pm 113.39
Quercetin	83.48 \pm 1.21	na	na
Gallic acid	na	73.89 \pm 1.93	na

na : not applicable.

americanum showed the scavenging percentage of 68.42 \pm 3.68%, whereas *P. lappacea* extracts showed 58.76

\pm 3.42%. Regarding hydrogen peroxide assay, both extracts showed moderate activity with 38.68 \pm 4.18 for

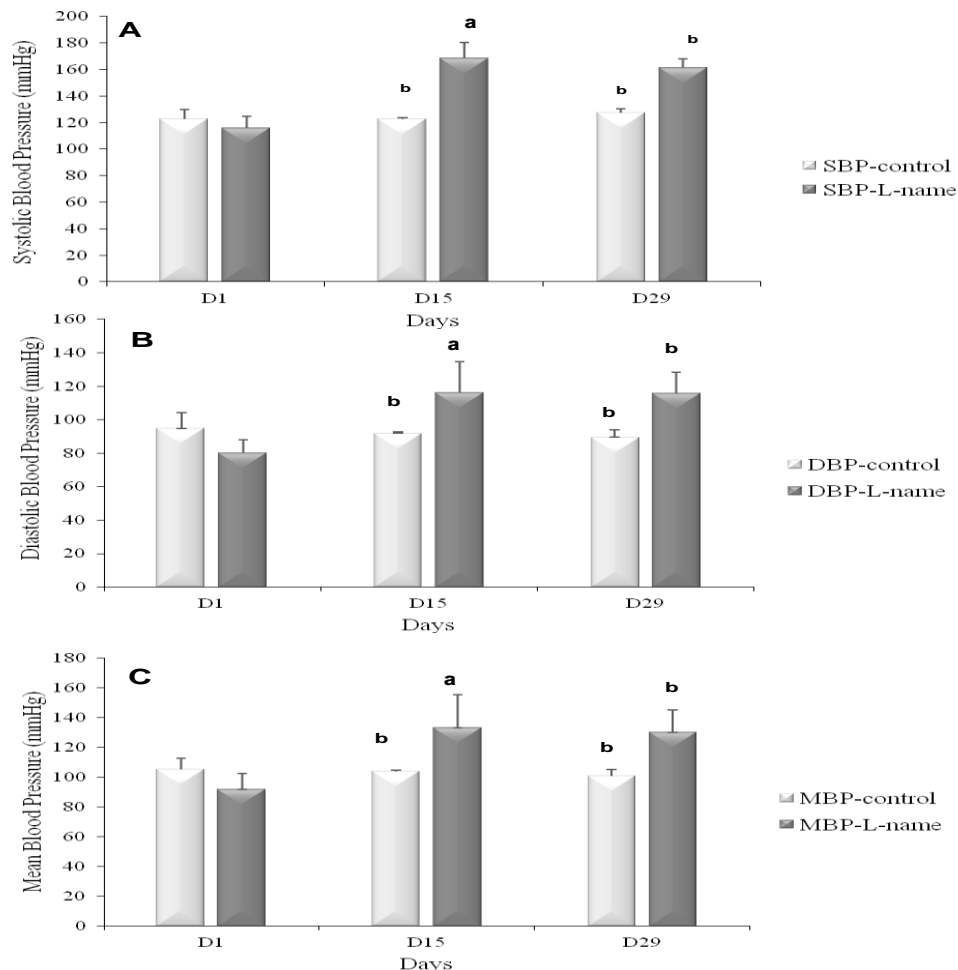


Figure 2. Effect of L-NAME on systolic (A), diastolic (B) and mean blood pressure (C). SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MBP: Mean blood pressure. Day 1 to 14: L-NAME administration; day 15 to 28: Distilled water administration. Data on day 15 were compared to those of day 1 to confirm induction of hypertension and perform statistical analyzes. Same procedures were used for the comparison of data between day 15 and 29. ^a ($p < 0.05$): significant change after L-NAME administration; ^b ($p > 0.05$): No significant change after L-NAME administration.

O. americanum against 32.67 ± 2.45 for *P. lappacea*.

Effect of extracts on blood pressure and heart rate

During the twenty-eight days, blood pressure was measured weekly using non-invasive method. No significant changes in mean blood pressure (MBP) were observed in the control group (105.2 ± 7.44 to 101 ± 4.4 mmHg) during the four weeks of experimentation. A significant increase of SBP, DBP and MBP was observed in animals that received L-NAME daily for fourteen (14) days as shown in Figure 2. Administration of OAE and PLE at 250 and 500 mg/kg body weight for fourteen days following L-NAME administration induced a significant decrease in blood pressure. OAE and PLE at 250 mg/kg bw induced significant decrease in SBP, DBP and MBP

of all the groups and there is no significant change between results obtained at 250 and 500 mg/kg bw. From day 15 to 29, *O. americanum* decreased MBP from 118.2 ± 00.32 to 76.80 ± 3.36 mmHg and *P. lappacea* from 143.0 ± 12.40 to 101.8 ± 8.24 mmHg. Similar results were obtained with losartan and captopril which respectively reduced blood pressure from 122.2 ± 06.16 to 83.00 ± 4.80 mmHg and 127.4 ± 03.52 to 87.20 ± 3.36 mmHg as presented in Tables 3 and 4. Contrary to blood pressure, administration of L-NAME (40 mg/kg/day) to animals for two weeks induced a significant decrease in the heart rate as shown in Table 5. Administration of OAE, PLE and reference drugs (Losartan and Captopril) restored the heart rate (HR). The significant increase ($p < 0.05$) of heart rate was observed at 500 mg/kg/bw as shown in Table 5.

Table 3. Effect of ethanolic extracts of *O. americanum* and *P. lappacea* on systolic and diastolic blood pressure in L-NAME-induced hypertensive Wistar rats.

Treatment	[C] (mg/kg.bw)	Systolic blood pressure			Diastolic blood pressure		
		Day 1	Day 14	Day 28	Day 1	Day 14	Day 28
Control (H ₂ O)	-	122.8 ± 7.04	122.8 ± 0.8	127.4 ± 3.04	94.8 ± 9.44	91.8 ± 1.04	89.4 ± 4.64
<i>O. americanum</i>	250	120.8 ± 4.25	156.8 ± 11.52 ^a	129.0 ± 06.16 ^a	73.8 ± 03.76	118.2 ± 00.32 ^a	76.80 ± 3.36 ^a
	500	124.6 ± 6.08	159.0 ± 08.40 ^a	118.0 ± 07.60 ^a	92.6 ± 07.04	101.2 ± 17.52 ^b	82.00 ± 7.60 ^b
<i>P. lappacea</i>	250	119.8 ± 8.64	179.2 ± 08.64 ^a	133.8 ± 12.24 ^a	79.8 ± 10.64	143.0 ± 12.40 ^a	101.8 ± 8.24 ^a
	500	131.4 ± 1.68	167.8 ± 09.44 ^a	140.0 ± 08.00 ^a	96.4 ± 02.88	121.4 ± 07.28 ^a	104.6 ± 6.72 ^a
Losartan	100	122.2 ± 1.92	168.0 ± 05.60 ^a	114.2 ± 04.32 ^a	91.0 ± 00.80	122.2 ± 06.16 ^a	83.00 ± 4.80 ^a
Captopril	100	115.6 ± 3.68	167.8 ± 04.16 ^a	116.8 ± 03.44 ^a	79.6 ± 04.72	127.4 ± 03.52 ^a	87.20 ± 3.36 ^a

Day 1 to 14: L-NAME administration; day 15 to 28: Extracts administration; [C] (mg/kg.bw): Concentration of extracts mg per kg of body weight of rats; ^a(p < 0.05): Significant change of SBP or DBP after L-NAME and extracts administration; ^b (p > 0.05): No significant change of DBP after L-NAME or extracts administration.

Table 4. Effect of ethanolic extracts of *O. americanum* and *P. lappacea* on mean blood pressure in L-NAME-induced hypertensive Wistar rats.

Treatment	[C] (mg/kg.bw)	Day 1	Day 15	Day 28
Control (H ₂ O)	-	105.2 ± 7.44	104 ± 0.8	101 ± 4.4
<i>O. americanum</i>	250	88.60 ± 3.12	138.4 ± 07.52 ^a	91.80 ± 6.56 ^a
	500	102.0 ± 5.60	119.0 ± 13.60 ^a	91.20 ± 5.04 ^a
<i>P. lappacea</i>	250	92.80 ± 9.84	154.4 ± 11.28 ^a	111.8 ± 9.44 ^a
	500	107.8 ± 2.24	136.4 ± 08.08 ^a	116.0 ± 7.20 ^a
Losartan	100	101.0 ± 0.80	135.8 ± 05.52 ^a	93.20 ± 4.72 ^a
Captopril	100	92.20 ± 3.84	136.0 ± 09.28 ^a	92.80 ± 1.84 ^a

Day 1 to 14: L-NAME administration; day 15 to 28: extracts administration; ^a (p < 0.05): Significant change of MBP after L-NAME and extracts administration.

Table 5. Effect of ethanolic extracts of *O. americanum* and *P. lappacea* on heart rate in L-NAME-induced hypertensive Wistar rats.

Treatment	[C] (mg/kg.bw)	Day 1	Day 14	Day 28
<i>O. americanum</i>	250	322.20 ± 10.24	261.40 ± 03.12 ^a	275.80 ± 25.76 ^b
	500	276.20 ± 35.84	249.20 ± 41.44 ^b	330.80 ± 12.64 ^a
<i>P. lappacea</i>	250	304.80 ± 11.44	211.80 ± 33.76 ^a	251.00 ± 37.60 ^b
	500	269.40 ± 32.88	182.80 ± 33.44 ^a	274.40 ± 40.48 ^a
Losartan	100	294.00 ± 36.80	205.00 ± 25.60 ^a	264.00 ± 22.00 ^a
Captopril	100	308.00 ± 28.00	247.00 ± 30.40 ^a	310.60 ± 02.72 ^a
L-NAME	40	266.00 ± 34.40	189.40 ± 42.64 ^a	304.80 ± 15.84 ^a
Control	-	277.80 ± 32.24	267.80 ± 36.24 ^b	307.40 ± 24.32 ^b

Day 1 to 14: L-NAME administration; day 15 to 28: Extracts administration. ^a (p < 0.05): Significant change of HR after L-NAME and extracts administration; ^b (p > 0.05): No significant change of HR after L-NAME and extracts administration.

DISCUSSION

In Benin, the use of medicinal plants for primary health care and the management of various diseases remains a reality. The management of hypertension does not escape this tradition and this has been confirmed by the results of recent surveys on the use of medicinal plants by hypertensives (Lagnika et al., 2016). In this study, we investigated the phytochemical constituents, total phenolic and flavonoids contents, antioxidant effect and the capacity of ethanolic extracts of *O. americanum* (OAE) and *P. lappacea* (PLE) to reduce blood pressure.

The phytochemical analysis of ethanolic extracts of OAE and PLE revealed the presence of various phytoconstituents. A large similarity was observed with previous published data about *O. americanum* (Birari and Dhulgande, 2010; Sarma and Venkata, 2011). However, differences were observed with coumarins (Dibala et al., 2016), alkaloids (Enemali and Udedi, 2018) and tannins (Elya et al., 2015). Regarding *P. lappacea*, a similitude was also observed with previous study (Udegbumam et al., 2014; Hoekou et al., 2012). Contrary to our results, alkaloids and tannins have been detected in ethanolic extract. These differences could be due to the phyto-geographical distribution, the phenology, the physiological stage of the species, the extraction method and/or solvents (Goli et al., 2005; Tarnaud et al., 2010).

A similarity was noted within secondary metabolites detected in OAE and PLE. The flavonoids, triterpenes, coumarins, essential oils, lignanes and anthocyanines are the secondary metabolites detected. Some of these phytoconstituents are well known for their antioxidant activity and their capacity to decrease high blood pressure (Oh et al., 2008). The antioxidant properties of tannins, flavonoids and coumarins make them protective molecules against free radicals that play an important role in the occurrence of more than 200 diseases such as cardiovascular diseases, cancer, hypertension, arthritis (Adjatin et al., 2013; Amoussa et al., 2015; Lajous et al., 2016; Bekoe et al., 2017). In general, flavonoids intake has been reported to have an inverse relation with cardiovascular disease and polyphenols have vascular protective effect (Siti et al., 2015).

Chronic administrations of L-NAME to rats significantly increase SBP, DBP and MBP when compared to control group. It is known that L-NAME administration causes a chronic increase in blood pressure in rats model (Gardiner et al., 1990; Babál et al., 1997). The increase in blood pressure could be explained by inhibition of nitric oxide (NO) synthesis by L-NAME (Kimura et al., 2017). Thus, a sufficient amount of NO is associated with normal vasodilatation and normal blood pressure, whereas inhibition of NO production may lead to hypertension (Nyadjeu et al., 2013; Sung et al., 2013). These data clearly justify the significant increase in SBP, DBP and MBP after L-NAME administration in our study. The increase in blood pressure during treatment with L-NAME

may be associated with NO deficiency and alterations in various blood pressure regulation systems. Many studies reported that chronic blockade of NO synthesis by NOS inhibitors like L-NAME lead to endothelial dysfunction, significant increase in blood pressure and further pathological injuries to the cardiovascular system and kidneys, which may lead to aggravation of hypertension (Graciano et al., 2004). Evidence have been also provided that chronic inhibition of NO synthesis in rats leads to elevations of systemic blood pressure and peripheral vascular resistance with alteration of vascular responsiveness. These vascular alterations were associated with marked oxidative stress (Veerappan and Senthilkumar, 2015). These data clearly justify the significant increase in SBP, DBP and MBP after L-NAME administration in our study.

Chronic oral administration of ethanol extracts of *O. americanum* and *P. lappacea* to L-NAME-induced hypertensive rats caused a significant decreased in SBP, DBP and MBP. Same results were observed for losartan and captopril used as standard drugs. The decrease of blood pressure by extracts may be associated with the regulation of oxidative stress associated with endothelial dysfunction after L-NAME administration. Previous reports showed that plant extracts containing flavonoids and/or triterpenes exert antihypertensive effects through the combination of the vasodilatory and antioxidant activities (Oh et al., 2008; Curin and Andriantsitohaina, 2005). Antioxidants are also reported to be beneficial in preventing endothelial dysfunction by scavenging superoxide and peroxynitrite (Kang et al., 2015). In this study, the analysis of ethanol extracts of *O. americanum* and *P. lappacea* allowed identification of phenolic acids and flavonoids which could contribute to their antioxidant and antihypertensive activity. The antioxidant activity of *O. americanum* (Shobo et al., 2015; Dinata et al., 2015; Enemali and Udedi, 2018) and *P. lappacea* (Apenteng et al., 2014; Prasad et al., 2014; Jazy et al., 2018) have been reported previously; thus, the decrease of MBP might be associated with the phenolic acids and flavonoids identified in extracts. Indeed, ferulic acid is one of these phenolic acids endowed with varied biological potential such as antioxidant, increase NO synthesis, free radical scavenger activity and vasodilatory effect (Kumar and Vikas, 2014; Drăgan et al., 2018). Chrysin have been showed to possess an antihypertensive effect by lowering blood pressure, lipid peroxides and improved antioxidant status. The suggested pharmacological mechanism is that chrysin inhibits production of superoxide and hydroxyl free radicals in enzymatic and nonenzymatic systems (Veerappan and Thekkumalai, 2018). Rutin identified in extract was showed to increase NO production in human endothelial cells and improved endothelial functions (Ugusman et al., 2014; Aditya and Ajay, 2017). It has also been reported that ellagic acid attenuates hypertension and possibly improving nitric oxide bioavailability. Likewise, caffeic acid and chlorogenic acid

decreased blood pressure and improved nitric oxide (NO) bioavailability by reducing activities of key enzymes linked to the pathogenesis of hypertension in cyclosporine-induced rats. These might be possible suggested mechanism of action of the phenolic acids and flavonoids identified in studied plants (Chiou et al., 2017; Agunloye et al., 2019).

Moreover, it is known that elevation of vasoconstriction and attenuation of vasorelaxation was observed in different parts of the vascular tree and increased sympathetic activity and alterations in renin-angiotensin system in L-NAME treated rats (Rossoni et al., 2007). The imbalance of vasoconstrictor and vasodilator systems caused an elevation of blood pressure in hypertension. Likewise, the reduction of NO dependent vasodilation (vasoconstriction) is the result of NO deficient hypertension which is followed by L-NAME administration (Zicha et al., 2006). Thus, the effect of plants studied might be associated with responses of balance between vasodilatation and vasoconstriction of blood vessels. Previous studies have demonstrated that ellagic acid improved vascular response affected by hypertension (Jordão et al., 2017). Several authors have also suggested that decrease in blood pressure and vasodilator effect could be created by phenolic acids such as tannic acid or ferulic acid (Turgut et al., 2015; Porter et al., 2010). Taking into account these phenolic acids identified in the ethanolic extracts of *O. americanum* and *P. lappacea*, we could suggest that the effect of OAE and PLE against high blood pressure could be explained by their vasorelaxant property (Drăgan et al., 2018). The antihypertensive effect of plants could also be due to their ability to reduce the peripheral resistance via their vasodilating activities (Sung et al., 2013; Bilanda et al., 2017). In this part, we could suppose that OAE and PLE had direct effect on vascular smooth muscle cells.

Inhibition of NO production by L-NAME may have increased the effect of reactive oxygen species (ROS) generated by vascular NADPH oxidase, resulting in endothelial dysfunction (Sung et al., 2013). NADPH oxidase is critically involved in increased blood pressure, O₂ production, vascular hypertrophy, inflammation and endothelial dysfunction in experimental and clinical hypertension (Lodi et al., 2006; Beswick et al., 2001). Previous report showed that administration of ellagic acid to L-NAME induce-hypertensive rats reduced blood pressure and attenuates hypertension by reducing NADPH oxidase subunit p47^{phox} expression, which prevents oxidative stress and restores NO bioavailability (Thewarid et al., 2015). The decrease in blood pressure after administration of OAE and PLE could be related to the inhibition of NADPH oxidase activity.

Conclusion

Overall, the present study demonstrated that OAE and PLE at a dose of 250 mg/kg bw exhibited antihypertensive

effect by lowered blood pressure. The interesting antioxidant and antiradicals potential and phenolic contents were described. The presence of phenolic acids and flavonoids contributed to the biological effects observed. These results may partially justify the traditional use of studied plants for the management of hypertension; however, further studies need to be done to determine the mechanism of action of the extracts and to consider the valorization of these plants as part of the management of hypertension.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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