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### RESEARCH ARTICLE

#### PRELIMINARY PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATIONS OF *SARCOCEPHALUS POBEGUINII* HUA EX PELLEGR (RUBIACEAE) STEM BARK EXTRACT AGAINST L-NAME-INDUCED HYPERTENSION IN RATS.

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#### Abstract

Hypertension is a chronic medical condition. Because of its high incidence and morbidity, various classes of drugs and regimens have been proposed for the control of hypertension including medicinal plant extracts. *Sarcocephalus pobeguini* is used by gabonese traditional healers to treat high blood pressure. We here by investigated the phytochemical constituents and preventive effects of the aqueous extract of *S.pobeguini* against N<sup>0</sup>-nitro-L-Arginine Methyl Ester (L-NAME) –induced hypertension in Wistar rats.

**Materials and Methods:** The plant extract was screened for the presence of various secondary metabolites by classic colorimetric methods. In preventive treatment, six groups of 5 rats each orally received: distilled water (10 mL/kg/day), L-NAME (50 mg/kg/day), L-NAME and extract (200 or 400 mg/kg/day), L-NAME and Esidrix (20 mg/kg/day), L-NAME and Furorese (40 mg/kg/day) during 4 weeks. At the end of the experimental period, blood pressure and heart rate were measured using the direct cannulation method. The effects of the plant extract were assayed using colorimetric methods.

**Results:** The phytochemical screening showed the presence of alkaloids, cardiac glycosides, and compounds phenolic. The plant extract at 200 and 400 mg/kg/day significantly prevented (13.65%) the increase in arterial blood pressure. Administration of the plant extract led to the prevention of lipid profile increase in serum in L-NAME-induced hypertensive rats. *S. pobeguini* extract (400 mg/kg) also significantly prevented the increase in ASAT (29.44%) and ALAT

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(12.98%). The extract ameliorates tissues biomarkers of oxidative stress.

**Conclusion:** These results demonstrate that the extract of *S. pobeguinii* can prevent L-NAME-induced hypertension and oxidative stress in rats.

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### Introduction:-

Hypertension is a chronic medical condition in which the blood pressure in the arteries is elevated. Hypertension is a social health problem and the most important risk factor for the development of severe cardiac diseases like ischemic heart disease, stroke, renal and heart failure. It is one of the main health problems worldwide, an important cause of cardiovascular deaths in the world and a 'silent-killer', because it is asymptomatic whose onset of complications is insidious associated to hypertension (Benowitz and al., 2001). Among the 972 million hypertensive adults, 333 million (34%) live in developed countries and 65.7% in developing countries (Kearney and al., 2005). In 2000, in Sub-Saharan Africa, more than 80 million of patients were identified as being affected by hypertension, and in 2025, this number is predicted to reach 150 million if nothing is done to fight this disease (Fourcade and al., 2007). In 2008, within the 1-1.5 billion of people that were affected by this disease, around 40% of adults aged of 25 and older were diagnosed with high blood pressure, while there were only 600 million people suffering from high blood pressure in 1980 (WHO, 2011, Zafar and al., 2015). In Africa, the problem of hypertension is its high prevalence (46% of adults aged over 25 years), its frequent underdiagnosis and the complications that are frequently associated with it (Addo and al., 2007).

However the management of hypertension without any side effects and with a maximum patient adherence remains a challenge for researchers. Thus, the use of plant extracts can be an alternative for the treatment of these pathologies. In view of the expansion of non-transmissible diseases, the WHOAFR/RC50/R3 in August 31<sup>st</sup>, 2000, encouraged African countries to elaborate regional strategies based on traditional medicine in order to undertake research on medicinal plants and to improve their optimal uses in health care systems (Eddouks and al., 2009). Many reports estimated that approximately 80% of the population of developing countries still relies on traditional medicine for their primarily health care (Farnsworth and al., 1985; Hostettman and Marston, 2002; WHO, 2002) because it is better tolerated by human body and induces less side effects (Talha and al., 2011; Singh and al., 2015). In some African countries such as Cameroon, Ghana, Mali, Nigeria and Gabon, the first line of treatment is the use of medicinal plants at home (WHO, 2003) due to the cost of medication, which is beyond the means of most people (Saslis-Lagoudakis and Clarke, 2012).

Among the medicinal plants prescribed by traditional healers, Rubiaceae is a large family of 630 genera and about 13,143 species can be found worldwide, especially in tropical and warm regions. Over 60 species are used for more than 70 medicinal indications including malaria, hepatitis, eczema, oedema, cough, hypertension, diabetes and sexual weakness. Many of these plants exhibited antimalarial, antimicrobial, antihypertensive, antidiabetic, antioxidant and anti-inflammatory activities. In the Sub-Saharan region, several cases of their traditional usages have been reported (Karou and al, 2011) and more particularly the examples of *Nauclea* and *Sarcocephalus* species, (Razafimandimbison, 2002).

*Sarcocephalus pobeguinii* (syn. *Nauclea pobeguinii*) can be mainly found in Gabon, Cameroon and Equatorial Guinea (Chris and Issembé, 2000). In Cameroon, in the Upper Nyong valley forest, its peels are used for the prevention of miscarriages and threat of abortion (Jiofack and al, 2009). In Gabon, where the Eveya habitat is located, leaves infusion of *Sarcocephalus pobeguinii* are used as febrifuge while bark maceration is indicated for urogenital infections and the gonorrhoea (Raponda, 1953). This species, in addition with *Nauclea diderrichii* [(syn. *Nauclea trillesii*) (Pierre Merrill) and *Sarcocephalus diderrichii* (De Wild)], are used in folk medicine for the treatment of hypertension or diabetes associated or not to hypertension. In Guinea Conakry, the leaves and the stem barks of *Sarcocephalus pobeguinii* are recommended for the treatment of diarrhea, dysentery, cholera and hypertension (Baldé and al, 2006). The decoction of its roots has antihelmintic effects (Keita and al, 1999). The decoction of its stem barks presents antiseptic and anti-infectious (Magassouba and al, 2007) properties. In the western Nigeria, roots from *Sarcocephalus pobeguinii* are added to a decoction mixture comprising stem barks and branches of *Vismia guineensis*, trunk of *Spathodea campanula*, stem defoliated of *Sorghum bicolor*, and roots of *Lawsonia inermis*. They are used orally against hypertension and sleep nightmare agitation/disorders. *S. pobeguinii* serves to prevent the unrests of delivery while the powder of these same parts is used against abdominal pains and

stomach aches in Senegal (Kerharo and Adam, 1974). In Sudan and North Kordofan, it acts as remedy against cough, hypertension and headaches (El-Kamali, 2009).

The chemical investigations from *S. pobeguinii* were focused on alkaloids and other chemical classes (Zeches, 1985; Anam, 1997; Xu, 2012; Agnanié, 2016). Besides, a few biological studies have also been conducted on this species for its anti-malarial, antiplasmodial, antiradical and antidiabetic activities (Mesia, 2005; 2010; Kahumu 2010; Mesia, 2011; 2012a; 2012b; Agnanié and al, 2016). The present study was designed to investigate the phytochemical and the preventive effect of the aqueous extract of *S. pobeguinii* in rat model of L-NAME induced hypertension.

## **Materials and methods:-**

### **Plant material and preparation of extract:-**

The stem barks of *Sarcocephalus pobeguinii* Hua ex Pellegr. were harvested in the outskirts of Lambaréné (District of the Ogooué and Lakes, Gabon) in January 2010. The plant material was identified by comparison with those of the National herbarium of the Institute of Pharmacopea and Traditional Medicine (IPHAMETRA) where voucher specimens were deposited under the following numbers, Azizet Issembé172 and Wilks 1035. The bark was dried at room temperature and reduced to a powder. The dried bark powder (100 g) was then macerated in 1000 mL of distilled water for fifteen minutes. The mixture was filtered and the filtrate was frozen then freeze-dried with a yield of 10.34%.

### **Animals:-**

The study was conducted on male Wistar rats aged of 3 months, weighing 160 to 220 g at the beginning of the experiment. They were obtained from the animal house of the Faculty of Sciences of University Yaoundé I, Cameroon and maintained under standard laboratory conditions (12/12 h light/dark cycle) and had free access to standard commercial diet and tap water. After two weeks of acclimation, thirty animals were divided into experimental groups. Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethical Committee (Reg. N° FWAIRD 0001954).

### **Phytochemical screening:-**

Phytochemical screening for the main chemical classes was undertaken using standard qualitative methods based on colorimetric and differential precipitations with reagents according to the methods of Odebiyi and Sofowora, 1978, Harbone, 1992; Evans, 1996; Houghton&Amala, 1998; Brain & Turner, 1985; Brain & Turner, 1985; Evans and al, 1996; Ciulei, 1981; Sofowora, 1993; Ciulei, 1981). The reagents following were used for the phytochemical tests: Dragendorff reagent, Neu reagent, Wagner reagents, Hager's reagents, ethyl acetate, ammonium chloride solution, Fehling's solution 1 & 2, sulphuric acid, ferric chloride, lead sub-acute, ethanol, water, million's reagent, picric acid, Molish reagent, iodine, Shinoda and Stiany reaction.

### **Experimental procedure for assayed preventive effect of the plant extract against L-NAME-induced hypertension:-**

To determine the antihypertensive activity of *Sarcocephalus pobeguinii* we used the classic method of hypertension induced by the N<sup>o</sup>-nitro-L-Arginine Methyl Ester (L-NAME) according to Badhyal and al, 2003; Bahgat and al, 2008; Nguelefack and al, 2008. Rats were randomly divided into six groups of five individuals. The first group received distilled water (10 mL/kg) and served as control, the second group received L-NAME at the dose of 50 mg/kg/day, the third and fourth groups were treated with L-NAME (50 mg/kg/day) plus the plant extract at doses of 200 and 400 mg/kg/day, respectively. The fifth and sixth groups received either esidrix (20 mg/kg/day) or furorese (40 mg/kg/day), in addition to L-NAME (50mg/kg/day). In the present study, we used diuretics as reference drugs instead of inhibitor of angiotensin converting enzyme (Thomas, 2000), because of the diuretic and natriuretic effect of NOS inhibitors commonly observed/previously reported at high doses, although this inhibitor would provide important information regarding blood pressure regulation (Liang and al, 2001). Different treatments were administered per os once a day for 4 weeks. Throughout the experiment, body weight was evaluated every day.

### **Blood pressure and heart rate measurements:-**

At the end of the experimental period (4 weeks), arterial blood pressure and heart rate were measured as described by Bopda and al, (2007). Briefly, the rat was anesthetized using an intraperitoneal injection of urethane 15% (1.5 g/kgbw). The trachea was exposed and cannulated to facilitate spontaneous breathing. The arterial blood pressure was measured from right carotid artery via an arterial cannula connected to a pressure transducer coupled with a

hemodynamic recorder Biopac Student Lab. (MP35) and a computer. The heart rate was measured at the same time and the tracing curves were generated and recorded.

#### **Serum biochemical analysis:-**

After hemodynamic measurements, rats were killed by exsanguinations and arteriovenous blood was collected for biochemical analysis. Serum was separated by centrifugation (3600 rpm for 15 min) and stored in eppendorf tubes at -4°C for the determination of lipid profile, hepatic and renal function markers. The determination of biochemical parameters was performed using standardized enzymatic colorimetric methods by measurement of the optical density of the reaction products at the corresponding wavelength with a spectrophotometer (Genesys 20Thermo Spectronic). The serum protein levels were determined according to Gornal and al, (1949). The total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and glucose levels were assayed using commercial diagnostic Kits Randox (SA 34130, United Kingdom). Alanine and Aspartate Aminotransferases (ALAT and ASAT) were determined using Cypress diagnostics kits (UV kinetic test IFCC. HBE07 Code) as described by Reitman and Frankel (1957) while creatinine was evaluated using Fortress diagnostics kits (United Kingdom).

#### **Evaluation of ionic profile:-**

The determination of the ionic profile in serum was also performed using standardized colorimetric methods as described by Bartels and al, (1972) by measuring the different optical density of the products at the corresponding wavelength with a spectrophotometer. Potassium, sodium and chloride levels were determined according to the manufacturer protocols described by Randox, Cypress, Fortress and SGMitalia.

#### **Oxidative stress markers assays:-**

After blood collection, heart, aorta, liver and kidney were dissected out weighed using a digital electronic balance (Mettler PL301) and crushed respectively in McEwen solution (heart and aorta) or Tris buffer (liver and kidneys) to obtain homogenates at 20%. After centrifugation at 10,000 x g for 30 minutes, the supernatant was collected and stored at -20°C for biochemical analysis. The concentration of Tissue protein was determined according to Gornal and al, (1949) using the Biuret reagent and bovine serum as a standard. Superoxide dismutase (SOD) and catalase (CAT) activities were respectively determined according to Misra and Fridovich (1972) and Sinha (1972). The SOD activity was expressed in U/mg protein and the CAT activity was determined by the difference in absorbance per unit.

#### **Statistical analysis:-**

The results were expressed as mean  $\pm$  S.E.M. (standard error of the mean). The statistical analysis was performed using One-way analysis of variance (ANOVA) and Duncan post hoc test for the comparison between the control group and treated animals. The value of  $P < 0.05$  was considered as statistically significant.

#### **Results:-**

##### **Phytochemical screening:-**

Basic alkaloids, C-anthracenosides, cardiac glycosides, carotenoids, coumarins, flavonoids, glycosides (oses and holosides), leucoanthocyan, saponosides, Steroids, tannins and triterpenoids were identified.

##### **Effect of *Sarcocephalus pobeguinii* on blood pressure, heart rate and the body weight of L-NAME-induced hypertensive rats:-**

Table 2 shows that L-NAME (50 mg/kg/day) significantly increased the systolic blood pressure and heart rate of rats, while the body weight of hypertensive rats does not change. Blood pressure increased from  $110.4 \pm 4.5$  to  $159.6 \pm 2.8$  mmHg ( $P < 0.001$ ) and heart rate increased from 395.6 to 419.7 beats/min. The *S. pobeguinii* (200 and 400 mg/kg/day) aqueous extract significantly ( $P < 0.001$ ) prevented this increase in mean arterial blood pressure L-NAME-induced hypertensive rats. At both doses of *S. pobeguinii*, there were no significant changes in heart rate of hypertensive rats, or in body weight. Reference drugs (20 mg/kg/day of Esidrix and 40 mg/kg/day of Furorese) significantly decreased blood pressure ( $P < 0.001$ ) as compared to the hypertensive group (Table 2).

**Table 2:-** Effect of *Sarcocephalus pobeguunii* on systolic blood pressure, heart rate and body weight of hypertensive rats.

	Water (Control)	L-NAME	L-NAME + Sp 200	L-NAME + Sp 400	L-NAME + Esidrix	L-NAME + Furorese
Systolic Blood pressure (mmHg),	110.4 ± 4.5	159.6 ± 2.8 <sup>c</sup>	137.8 ± 2.9 <sup>by</sup>	138.4 ± 2.5 <sup>y</sup>	139.4 ± 3.4 <sup>ay</sup>	141.7 ± 2.4 <sup>y</sup>
Heart rate (Beats/min)	395.6 ± 6.1	419.7 ± 6.6 <sup>c</sup>	364.9 ± 8.4	345.4 ± 7.3	394.6 ± 9.7	373.3 ± 4.9
Body Weight (g)	194.0 ± 2.2	194.0 ± 3.9	197.0 ± 2.1	196.0 ± 2.5	182.0 ± 1.6	176.0 ± 1.5

Values are expressed as means ± S.E.M. of 5 rats; <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.001, significantly different as compared to the control group; <sup>y</sup>P < 0.001, significantly different as compared to the L-NAME group. Sp = aqueous extract of *Sarcocephalus pobeguunii*.

### Effect of *Sarcocephalus pobeguunii* on lipid profile and glucose levels of hypertensive rats after 4 weeks of treatment:-

As shown in Table 3, the administration of L-NAME during 4 weeks has significantly increased the total cholesterol by 28.59%, LDL-cholesterol by 90.04%, triglycerides by 19.61%, and glycaemia by 11.91% as compared to normal untreated rats. Co-administration of L-NAME (50mg/kg)-Esidrix (20 mg/kg/day) or-Furorese (40 mg/kg/day) or L-NAME (50mg/kg) plus *S. pobeguunii* (200 or 400mg/kg) significantly inhibited the increase of these parameters in L-NAME-induced hypertensive rats.

**Table 3 :-** Effect of *Sarcocephalus pobeguunii* on the lipid profile and glucose rate of hypertensive rats.

	Water (control)	L-NAME	L-NAME + Sp 200	L-NAME + Sp 400	L-NAME + Esidrix	L-NAME +Furorese
TG (mg/dL)	74.5 ± 3.0	89.1 ± 4.8 <sup>b</sup>	58.1 ± 2.8 <sup>ab</sup>	70.3 ± 4.0 <sup>by</sup>	74.7 ± 4.8 <sup>ca</sup>	81.3 ± 3.4 <sup>bp</sup>
Chol (mg/dL)	76.1 ± 4.5	97.8 ± 4.1 <sup>y</sup>	71.6 ± 3.0 <sup>cy</sup>	82.4 ± 5.1 <sup>cy</sup>	85.9 ± 1.2 <sup>cy</sup>	89.1 ± 4.3 <sup>cy</sup>
LDL-Chol (mg/dL)	28.1 ± 1.3	53.5 ± 2.6 <sup>c</sup>	46.3 ± 4.9 <sup>cy</sup>	44.6 ± 2.7 <sup>cy</sup>	35.3 ± 6.7 <sup>cy</sup>	30.0 ± 2.3 <sup>cy</sup>
HDL-Chol (mg/dL)	6.7 ± 0.3	5.5 ± 0.5	8.9 ± 0.4 <sup>cy</sup>	9.1 ± 0.3 <sup>cy</sup>	8.2 ± 0.4 <sup>cy</sup>	9.3 ± 0.5 <sup>cy</sup>
Glucose (mg/dL)	89.0 ± 0.6	99.6 ± 0.5 <sup>c</sup>	93.7 ± 1.8 <sup>cy</sup>	92.0 ± 2.8 <sup>cy</sup>	95.7 ± 2.7 <sup>cy</sup>	91.5 ± 1.3 <sup>cy</sup>

Values are expressed as means ± S.E.M. of 5 rats; <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.001, significantly different compared to the control group; <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, <sup>y</sup>P < 0.001, significantly different compared to the L-NAME group. Sp = aqueous extract of *Sarcocephalus pobeguunii*. TG= triglycerides; chol=cholesterol

### Effect of *Sarcocephalus pobeguunii* on some parameters of liver and kidney functions:-

The results presented in Table 4 show that the concentrations of proteins, creatinine, sodium, potassium, chlorine and ASAT and ALAT activities increased in serum of hypertensive rats. The plant extract (200 and 400 mg/kg/day), Esidrix (20 mg/kg/day) or Furorese (40 mg/kg/day), administered simultaneously with L-NAME (50 mg/kg), blunted the increase in ALAT and ASAT activities and protein, sodium and potassium levels.

**Table 4:-**Effect of *Sarcocephalus pobeguunii* on the liver and kidney functions of hypertensive rats.

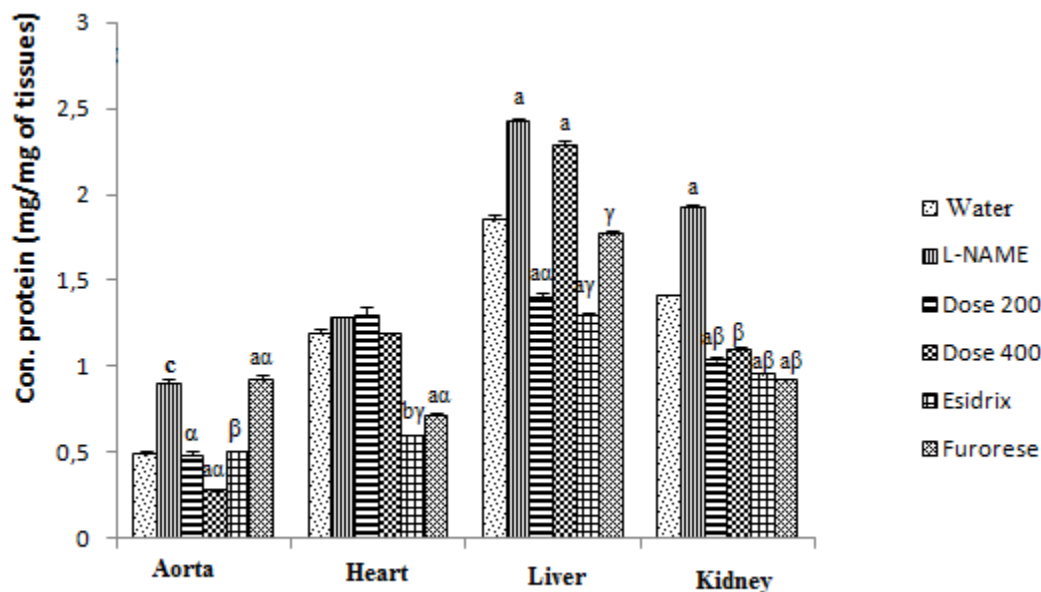
	Water	L-NAME	L-NAME + Sp 200	L-NAME + Sp 400	L-NAME + Esidrix	L-NAME + Furorese
Proteins (mg/dL)	3.6 ± 0.1	8.0 ± 0.1 <sup>c</sup>	5.2 ± 0.1 <sup>a</sup>	5.4 ± 0.1 <sup>ba</sup>	5.3 ± 0.1 <sup>ba</sup>	4.44 ± 0.0 <sup>aa</sup>
ASAT (UI)	31.3 ± 2.9	48.7 ± 1.8 <sup>c</sup>	43.8 ± 3.7 <sup>cy</sup>	34.3 ± 2.9 <sup>cy</sup>	39.7 ± 1.6 <sup>cy</sup>	37.5 ± 1.2 <sup>cy</sup>
ALAT (UI)	22.6 ± 1.8	27.04 ± 2.4 <sup>a</sup>	23.7 ± 2.1 <sup>by</sup>	23.8 ± 1.3 <sup>ay</sup>	24.2 ± 2.8 <sup>cy</sup>	24.8 ± 1.0 <sup>cy</sup>
Creatinine (mg/dL)	1.3 ± 0.0	1.5 ± 0.0 <sup>aa</sup>	1.3 ± 0.0	1.4 ± 0.0	1.2 ± 0.1 <sup>ab</sup>	1.2 ± 0.1
Sodium (mmol/L)	72.4 ± 2.3	86.4 ± 3.0 <sup>c</sup>	83.6 ± 2.9 <sup>cy</sup>	75.1 ± 3.3 <sup>cy</sup>	79.9 ± 2.5 <sup>cy</sup>	88.3 ± 1.5 <sup>c</sup>
Potassium	4.7 ± 0.2	9.6 ± 0.4 <sup>c</sup>	8.9 ± 0.3 <sup>cy</sup>	6.5 ± 0.1 <sup>cy</sup>	5.7 ± 0.2 <sup>cy</sup>	6.0 ± 0.5 <sup>cy</sup>

(mmol/L)						
<b>Chlore (mmol/L)</b>	62.5 ± 2.1	90.2 ± 3.6 <sup>c</sup>	90.7 ± 2.8 <sup>c</sup>	77.05 ± 3.1 <sup>αβ</sup>	94.1 ± 1.5 <sup>c</sup>	90.9 ± 1.9 <sup>c</sup>

Values are expressed as means ± S.E.M. of 5 rats; <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.001, significantly different compared to the control group; <sup>α</sup>P < 0.05, <sup>β</sup>P < 0.01, <sup>γ</sup>P < 0.001, significantly different compared to the L-NAME group. Sp = aqueous extract of *Sarcocephalus pobeguinii*.

#### Effect of *Sarcocephalus pobeguunii* on tissues proteins levels of hypertensive rats after 4 weeks of treatment:-

Figure 1 shows that the proteins concentrations significantly increased in the aorta, liver and kidneys of the rats treated with L-NAME (Figure 1). The administration of *S. pobeguunii* (200 and 400 mg/kg/day) or Esidrix (20 mg/kg/day), significantly decreased the protein level in the aorta and the kidneys. Furorese significantly inhibited the increase in protein levels of hypertensive rats in the heart and kidneys.



**Fig. 1:-** Effect of *Sarcocephalus pobeguunii*, Esidrix and Furorese on tissue protein levels of L-NAME induced hypertensive rats after 4 weeks of treatment.

Each bar represents a mean ± S.E.M. of 5 rats; <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.001, significantly different compared to the control group; <sup>α</sup>P < 0.05, <sup>β</sup>P < 0.01, <sup>γ</sup>P < 0.001, significantly different compared to the L-NAME group. Sp = aqueous extract of *Sarcocephalus pobeguunii*, Esi = Esidrix, Fur = Furorese.

#### Effect of *S. pobeguunii* on some parameters of oxidative stress :-

##### Effect of *S. pobeguunii* on SOD activity:-

Animals receiving only L-NAME during 4 weeks, showed a significant increase in SOD activity by 161.72%, 139.05%, 127.12% and 120.24%, respectively, in the aorta, heart, liver and kidneys compared to normal control (Figure 2).

The administration of the plant extract induced a significant decrease in SOD activity by 39.37 % (p < 0.001) in aorta, 18.17% (p < 0.01) in liver and 55.48 % in kidneys (p < 0.01), at the dose of 200 mg/kg and by 20.36 % (p < 0.001), 23.65 % (p < 0.05), and 47.55 % (p < 0.05), respectively, in the aorta, liver and kidneys at the dose of 400 mg/kg.

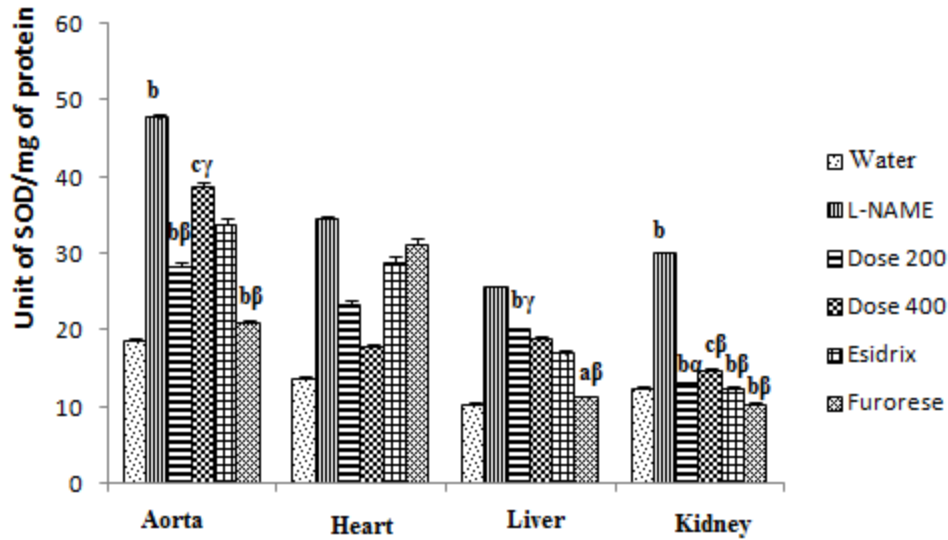


Fig. 2:-Effect of *Sarcocephalus pobeguunii*, Esidrix and Furorese on tissues SOD activity of L-NAME induced hypertensive rats after 4 weeks of treatment.

Each bar represents a mean ± S.E.M. of 5 rats; <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.001, significantly different compared to the control group; <sup>β</sup>P < 0.01, <sup>γ</sup>P < 0.001, significantly different compared to the L-NAME-treated group. *S<sub>p</sub>* = aqueous extract of *Sarcocephalus pobeguunii*, *Esi* = Esidrix, *Fur* = Furorese.

**Effect of *Sarcocephalus pobeguunii* on tissues catalase activity of hypertensive rats after 4 weeks of treatment:-**

Figure 3 shows a significant increase in catalase activity in tissues (aorta, heart, liver and kidneys) of hypertensive rats. However, the treatment of rats with *S. pobeguunii* (200 and 400 mg/kg/day), Esidrix (20 mg/kg/day) or Furorese (40 mg/kg/day) significantly decreased the activity of catalase in heart (Figure 3).

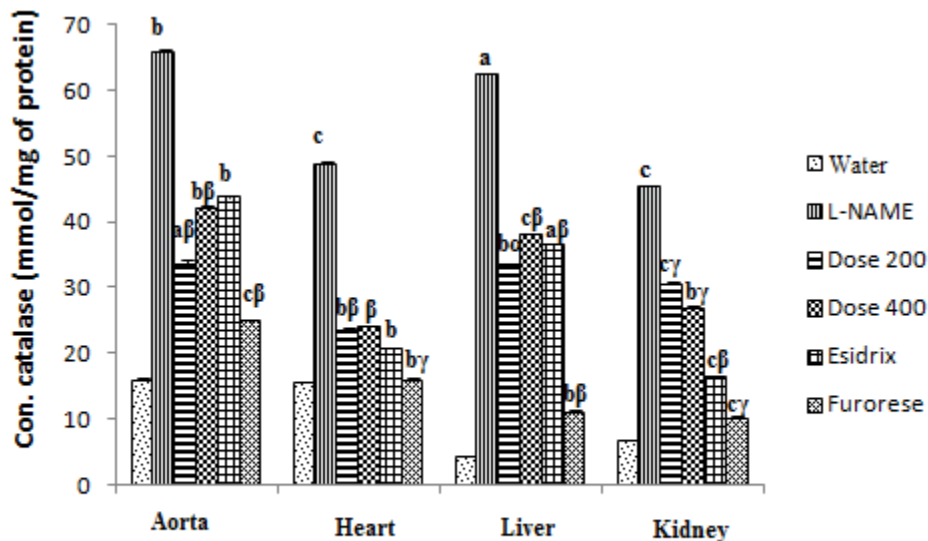


Fig. 3:-Effect of *Sarcocephalus pobeguunii*, Esidrix and Furorese on tissues activity of catalase on L-NAME-induced hypertensive rats after 4 weeks of treatment.

Each bar represents a mean  $\pm$  S.E.M. of 5 rats; <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.001, significantly different compared to the control group; <sup>b</sup>P < 0.01, <sup>y</sup>P < 0.001, significantly different compared to the L-NAME group. Sp = aqueous extract of *Sarcocephalus pobeguini*, Esi = Esidrix, Fur = Furorese.

### Discussion:-

The present study investigated the phytochemical and antihypertensive effect of the aqueous extract of the stem bark of *Sarcocephalus pobeguini* in L-NAME-induced hypertension in rats. The results of our preliminary phytochemical study of *S. pobeguini* revealed the presence of alkaloids, flavonoids, tannins, coumarins, carotenoids, anthracenoides, cardiogenic heterosides, steroids and triterpenoids. The presence of some of these compounds has been previously identified by other researchers (Zeches and al, 1985; Anam, 1997; Xu and al, 2012; Agnani and al, 2016) in *S. pobeguini*.

The administration of NOS inhibitor, L-NAME, results in increased blood pressure and ROS (reactive oxygen species) mediated tissue damage. These findings are in agreement with previous studies (Fortepiani and al, 1999, Peotta and al, 2001, Biancardi and al, 2007). The increase in arterial blood pressure is due to an increase in the sensitivity of arterial baro receptors and to the inhibition of NO synthesis which leads to vasoconstriction. The latter has been associated with enhanced sodium and water renal reabsorption (Fortepiani and al, 1999), renal vasoconstriction, elevation in oxidative stress (Duarte and al, 2002, Ndiya and al, 2003) and enhanced calcium signaling in smooth muscle cells. The administration of L-NAME (50 mg/kg/day) to rats for 4 weeks significantly increased blood pressure in our study, without any significant change in heart rate and body weight. Chronic inhibition of nitric oxide (NO) synthesis has been shown to result in arterial hypertension and an important blunting of the pressure diuresis and natriuresis response (Fortepiani and al, 1999). In our study, Esidrix and Furorese, diuretic drugs, taken as hypotensive drugs reference, like *S. pobeguini* extract, significantly decreased blood pressure in L-NAME-induced hypertensive rats. The aqueous extract is efficient by preventing the increase of blood pressure in L-NAME-induced hypertensive rats. These antihypertensive effects of *S. pobeguini* extract are comparable to those of Metchi and al, (2013) on the same type of hypertension using the extract of *Vitex cincinnensis* at the same doses of 200 and 400mg/kg. According to Khan and Gilani (2008), the extract of *Terminalia bellirica* consisting of flavonoids, sterols and tannins as *S. pobeguini*, causes a reduction in blood pressure of rats pre-treated with L-NAME and induced *in vitro* the relaxation of rats isolated aorta in the absence of endothelium. Perez-Vazcaino and al, (2009), reported that the injection of quercetin which is a flavonoid causes the decrease in blood pressure of rats pre-treated with L-NAME. Indeed, polyphenolic compounds are known for their antihypertensive and cardioprotective effects (Francisco and al, 2009; Davide and al, 2010). Rupasinghe and al, (2011) demonstrated that flavonoids could inhibit angiotensin converting enzyme. Angiotensin II (Ang II) is a key factor that causes vasoconstriction, salt retention and inflammation (Passaglia, 2015). The work of Eckly and Lugnier (1994), also reported that polyphenols (such as flavonoids) increase the production of cAMP and cGMP which cause the stimulation of certain potassium channels and would result in a relaxation of the aortic rings without endothelium, causing hyperpolarization of these vessels. This might explain the reduction of blood pressure in hypertensive rats since hyperpolarization causes the decrease in the concentration of calcium cytoplasmic ion through inhibition of calcium channels involved in the mechanism of vascular smooth muscle contraction.

L-NAME in this study induced a significant increase of the serum levels of total cholesterol, LDL-cholesterol, and triglycerides. These findings are in agreement with previous results which revealed that hypercholesterolemia and dyslipidemia are associated with the pathogenesis of hypertension induced by chronic L-NAME intake. Administration of the aqueous extract of *S. pobeguini* provided a beneficial action on rat's lipid profile with regard to the reduction of total cholesterol, LDL-cholesterol and triglycerides. The lipid lowering potential of the extract may be attributed to the presence of phytochemical constituents such as flavonoids, saponins, and tannins (Olagunju and al, 1995). Flavonoids are reported to lower LDL-cholesterol concentrations in hypercholesterolemia animals (Patel and al, 2009). Saponins have been shown to possess blood cholesterol lowering activity by inhibiting the intestinal reabsorption of cholesterol. Similarly, tannins are recognized for their ability to inhibit lipid absorption (Ravichandiran and al, 2012). Hence, the lipid lowering activity of the extract might also be due to the presence of these secondary metabolites, each with a single or diverse range of biological activities. Thus, the observed significant reduction in serum total lipids, total cholesterol and LDL cholesterol by the extract which can be ascribed to the phytochemical constituents of the stem bark implies that it can be used to prevent cardiovascular complications arising from hyperlipidemia.



Oxidative stress due to the production of large amounts of reactive oxygen species (ROS) is also considered as one of the most important factors involved in the pathogenesis of L-NAME-induced hypertension (Sainzand al, 2005). In the present work, antioxidant properties of the aqueous extract of *S. pobeguinii* were determined by measuring SOD and catalase activities in the tissues of rats induced by NOS inhibitor, L-NAME. Treatment with L-NAME in our study results in a significant increase in free-radical-scavenging enzymes (SOD and catalase) levels in tissues. Our data have been in accordance to the results of other researchers (Sainzand al, 2005, Kukongviriyapanand al, 2013). It has been reported that high-dose L-NAME treatment (40 to 50 mg/kg/day) increases levels of oxidative stress markers such as vascular superoxide ( $O_2^{\bullet-}$ ), plasma malondialdehyde (MDA) and plasma protein carbonyl (Nakmareongand al, 2011). In the L-NAME-induced hypertension model, it was suggested that a large quantity of superoxide production suppressed nitric oxide bioavailability (Pechanovaand al, 1999; Torokand al, 2008). In addition, Duarte and al, (2002) found an increase in tissues catalase levels in L-NAME hypertensive rats, indicating the involvement of oxidative stress in this animal model. *S. pobeguunii* extract significantly reduced the levels of SOD and catalase production as compared to rats treated with L-NAME alone, which suggested the antioxidant potential of *S. pobeguunii* aqueous extract against injury caused by free radicals. The ingestion of the extract in this study maintained the serum and tissue protein levels around the normal, probably by its antioxidant character.

We also examined the role of an increased haemodynamic parameters on the development of renal and liver damage in L-NAME-induced hypertension in rats. In the present study, the increase in serum creatinine after L-NAME treatment suggests compromised renal function in L-NAME-induced hypertension rats. Interestingly, the elevation in serum creatinine was accompanied by an increase in serum sodium, potassium and chloride levels suggesting functional damage of kidneys in L-NAME-induced hypertension. In animals treated with L-NAME, *S. pobeguunii* extract significantly prevented the development of kidney functional damage. It is well known that hypertension progressively alters the hepatic function (Gokcimen and al, 2007). In this study, we also investigated the protective effects of the plant extract against L-NAME-induced liver damage on relevant serum biomarkers (ALAT, ASAT and total protein). We have found that L-NAME treatment has led to an increased serum ALAT and ASAT activity. ALAT and ASAT are the most sensitive biomarkers directly implicated in the extent of hepatic damage and toxicity. The increase in serum enzymes activity in the L-NAME-treated group may be attributed to a generalized increase in membrane permeability, as reported by Shaarawyand al, (2009). This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation (Naikand al, 2007), indicating an inflammatory reaction. The significant decrease in liver biomarkers (ALAT, ASAT and serum proteins) in L-NAME and *S. pobeguunii* treated groups as compared with L-NAME control animals suggests a protective effect of our plant extract.

### **Conclusion:-**

This study revealed the beneficial effects of the aqueous extract of *S. pobeguunii* in the management of L-NAME-induced hypertension and improvement of lipid profile and oxidative stress. These findings could therefore justify its use in folk medicine in Gabon in the treatment of hypertension. However, further pharmacological and biochemical investigations are underway to elucidate the mechanism of antihypertensive effects of *S. pobeguunii*.

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