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**Original Article**

# Antihypertensive Effects of *Gmelina Arborea* Roxb (Verbenaceae) Leaves Crude Aqueous Extract Fractions in Wistar Rats

***[1]Awede B., [1]Osseni R., [2]Lagnika L., [3]Adjagba M., [1]Kpadonou C., [3]Darboux R. B. and [3]Laleye A.**

[1] Unité de Physiologie, Faculté des Sciences de la Santé, Université d'Abomey-Calavi, Benin.

[2] Unité de Biochimie et de Biologie moléculaire, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, Benin.

[3] Unité de Biologie Humaine, Faculté des Sciences de la Santé, Université d'Abomey-Calavi, Benin.

## Abstract

The present work was carried out to investigate the antihypertensive properties of fractions *Gmelina arborea* aqueous extract, one of the medicinal plants used in the treatment of arterial hypertension. Fractions of *Gmelina arborea* leaves were obtained by liquid-liquid extraction and by sephadex gel chromatography. The chemical screening and detection of anti-oxidant activity of extracts and fractions were performed by thin layer chromatography. Antihypertensive effect has been studied by administration of the extracts and fractions at the dose of 30mg/kg of body weight to wistar rats made hypertensive by L-NAME treatment. Rat arterial blood pressure was measured by carotid catheterization. Eight fractions named F1, F2, F3 and E1, E2, E3, E4, E5 were obtained. Altogether, flavonoïds, tannins, saponins, alkaloids, anthracens, napthtoquinons and coumarins were detected in the fractions. Except F3 fraction, all the other extracts and fractions have shown antioxydant activity. F2 fraction, E1 and E3 extracts induced significant reduction of rat mean arterial pressure from 165.5 ± 2.7mm Hg to respectively 121± 1,1mm Hg, 128.5± 2,8mm Hg and 134.2±4 mm Hg. These data suggest that the antihypertensive activity of the leaves of *Gmelina arborea* could be related to flavonoids compounds alone or synergistically with alkaloids and tannins compounds.

**Keywords:** High blood pressure**,** *gmelina arborea,* antioxidant, traditional medicine.

* Corresponding author: Unité de Physiologie, Faculté des Sciences de la Santé, Université d'Abomey-Calavi, Benin.
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## Introduction

High blood pressure, the major risk factor of cardiovascular diseases is an important health problem worldwide because of its prevalence and its multiple complications. While hypertension was, once, rare in sub-Saharan Africa, in the past decades, a progressive increase of its prevalence rate has been observed in Africa. From 19.7% in 1980, the overall prevalence of hypertension in Africa has been estimated to 27.4% in 2000 and 30.8% in 2010 (ADELOYE and BASQUIL, 2014). Hypertension prevalence, higher than 40%, has been reported in adult population of African countries such as Gambia and Sierra Leone (AWAD *et al.*, 2014).

In Benin, in 2008, it has been shown that 28.7% of the adult population suffers from arterial hypertension (Houinato *et al.*, 2012). The therapeutic management of hypertension is a long term and expensive process especially for developing countries population. In Benin, according to a study published in 2004, the cost of hypertension treatment represents almost 50% of the guaranteed minimum wage (Agboton *et al.*, 2004). Because of this situation, the affected population has gradually found an alternative to conventional drugs using medicinal plants.

Unfortunately, scientific evidence for safety, quality and efficacy has been shown for only few of the African medicinal plants (Diallo *et al.*, 2010). Among these medicinal plants, *Gmelina arborea* is one that the biological properties still have to be proven. In a preliminary study, an antihypertensive effect of the crude aqueous extract of *Gmelina arborea* have been observed in rats (unpublished personal data). The present study aimed at identifying the active fraction of the crude aqueous extract of *Gmelina arborea* leaves on hypertension induced in rats by administration of L-NAME.

## Materials and Methods

### *Vegetal Material*

Fresh leaves of *Gmelina arborea* were harvested at Zè (South of Benin) and authenticated at the National Herbal Section of Benin. Leaves were dried under the lee of sun at 20-25°C during ten (10) days. The dried leaves were pulverized and the powder was stored at room temperature until use. 150 g of the dried powder of *Gmelina arborea* were extracted at 80°C with 500ml of distilled water during 30 minutes. The decoction was filtered using Whatman paper N° 1 (Whatman international Ltd; Maidstone, England) and then concentrated by evaporation at 80°C under reduced pressure using a rotary evaporator Rotavapor Buchi R-3 (Sigma-Aldrich, Germany). The dried crude aqueous extract obtained was stored in at 4°C.

### *Liquid-Liquid Extraction*

20 g of the aqueous extract were dissolved with 500 ml of distilled water and then introduced in a 2L conical flask. A first separation was carried out with dichloromethane (700ml three times) and successively with ethyl acetate and n-butanol. The organic phases were recovered and evaporated at 30°C on a rotary evaporator. The residual aqueous phase was divided into two layers: one more pasty (mucilage) and the other more liquid and blackish. They were also evaporated at 70°C on the rotary evaporator under reduced pressure.

### *Fractionation on Sephadex LH- 20 Gel*

50 g of Sephadex LH-20; 25 – 100 µm (Merck, Belgium) gel was soaked in ethanol (elution solvent) for 48h before the beginning of fractionation. The column was delicately filled with the gel up to 2/3. Then, 5g of the crude aqueous extract were dissolved in ethanol/water (50/50) and fractioned on the Sephadex gel. 250 ml of ethanol were firstly used to wash the column three times and followed by a mixture of acetone/water.

### *Phyto-Chemical Analysis*

Seven different phytochemical groups were investigated. This phytochemical analysis was performed using the thin layer chromatography described by Wagner and Bladt (2001). 5 mg of each extract were dissolved in 1mL of an appropriate solvent (mixture of methanol/water (1: 1), dichloromethane or ethyl acetate). Chromatographic plates were then loaded with 10 µL of each extract and the migration was performed using appropriate system according to each chemical group.

### *Anti-Oxidant Activity*

A qualitative free radical scavenging activity was checked out by chromatographic method as described previously (Adjagba *et al.,* 2015). The extracts and fractions were spotted on the plate and after migration in appropriate solvent the plate was sprayed with 1, 1-diphenyl-2-pycrylhydrazyle (DPPH). The presence of antioxidant substances is revealed by apparition of a yellowish color in a purple bottom.

### *Animal Experiments*

*Experimental Groups*

Wistar rats (200 - 250g of body weight) were used for *in vivo* tests. The rats were housed in cages and maintained in a light controlled environment (12:12h light-dark cycle) and had unlimited access to food and water. They were assigned to eleven (11) groups of eight rats: one group of rats which received distilled water from day 1 to day 14 (control group) and ten groups of rats which were made hypertensive by administration of (N(G)-Nitro-L-Arginine Methyl Ester (L-NAME) at 20 mg/Kg of body weight for the 7 first days and which received specific treatment from day 8 to day 14 as follows:

*L-NAME Groups*

Rats were treated with L-NAME from Day 1 to Day 7 and received distilled water from day 8 to day 14.

*Eight (8) Groups*

In these groups, following 7-day-administration of L-NAME, rats of each group were treated with one of the extracts or fractions of *Gmelina arborea* (30 mg/kg of body weight) for 7 days.

*Captopril Groups*

animals in this group were treated with Captopril at 100mg/kg of body weight from day 8 to day 14 after L-NAME administration from day 1 to day 7.

All substances were orally administrated (gavage) to rats.

### *Blood Pressure Measurement*

At the end of the treatment, rats were anesthetized by intra peritoneal injection of thiopenthal (40mg/Kg of body weight) and their blood pressure were measured by catheterization of carotid artery as previously described (Awede *et al.*, 2010).

## Statistical Analysis

Blood pressure data were presented as mean ± SEM. Analysis of Variance (ANOVA) followed by a post-test of Bonferroni was used for comparison between groups. Statistical significance was set at p-value < 0, 05.

## Results

### *Fractions and Extracts*

Eight fractions were obtained of which we have five E1; E2; E3; E4; E5 by liquid-liquid extraction and three F1, F2; F3 by Sephadex gel chromatography. As shown in table 1, in liquid-liquid extraction, the aqueous extracts (E4; E5) proportion is more important (74.35%) than that of the organic phase (20.40%) while during the column chromatography, ethanol was considered as the best elution solvent (38.4%).

**Table 1:** *Gmelina arborea* fractions and extraction rate of the liquid-liquid partition and of the sephadex gel chromatography.

| Fractions | Extraction rate (%) |
|---|---|
| E1 | 0.24 |
| E2 | 1.60 |
| E3 | 2.90 |
| E4 | 58.86 |
| E5 | 25.56 |
| F1 | 21.6 |
| F2 | 16.8 |
| F3 | 10.0 |

E1, E2, E3, E4, and E5 represent respectively dichloromethane, ethyl acetate, butanol, aqueous and aqueous micellar fractions obtained by liquid liquid partition.
F1, F2 and F3 represent respectively ethanol first fraction, ethanol second fraction and acetone fraction obtained by sephadex gel chromatography.

### *Phytochemical Screening*

Chemical groups found in the fractions are summarized in Table 2. The phytochemical screening revealed the presence of coumarins, flavonoids, saponins, anthracenes and tannins in F2, E2 and E3. Only flavonoids were detected in E4 and E5 while flavonoids and naphtoquinons were detected in E1. Flavonoids, saponins and tannins were found in F1 fraction. The presence of alkaloids was detected in only E2 and E3 extracts. No chemical group investigated was detected in F3 fraction.

**Table 2:** Phytochemical screening of fractions of *Gmelina arborea* leaves crude aqueous extract

| Chemical groups | F1 | F2 | F3 | E1 | E2 | E3 | E4 | E5 |
|---|---|---|---|---|---|---|---|---|
| Coumarins | - | ++ | - | - | ++ | ++ | - | - |
| Naphthoquinones | - | - | - | + | - | - | - | - |
| Flavonoids | + | ++ | - | + | +++ | +++ | + | + |
| Saponins | + | ++ | - | - | +++ | ++ | - | - |
| Anthracenes | - | + | - | - | ++ | ++ | - | - |
| Alkaloids | - | | - | - | ++ | + | - | - |
| Tannins | + | ++ | - | - | ++ | ++ | - | - |

F1, F2 and F3 represent respectively ethanol first fraction, ethanol second Fraction and acetone fraction obtained by sephadex gel chromatography.
E1, E2, E3, E4, and E5 represent respectively dichloromethane, ethyl acetate, butanol, aqueous and aqueous micellar fractions obtained by liquid liquid partition.
(+) weakly detected; (++) detected; (+++) abundantly detected; (-) absent.

### *Anti-Oxidant Effect of the Extracts and Fractions of Gmelina Arborea*

Except F3 fraction, all the fractions had a radical scavenging activity but at different degrees. E1, E2 and F2 have shown the best activity.

### *Effects of Fractions on Rat Arterial Pressure*

7-day-administration of L-NAME increased the rat mean arterial pressure (MAP) from 134.8± 2.7 mmHg to 165.5±2.7 mmHg. As shown in figure 1, captopril administration induced a decrease of MAP to 127.5±3.8 mmHg. Among the three fractions F1, F2, F3 obtained by Sephadex gel chromatography, only F2 significantly reduced the arterial blood pressure from 165.5±2.7 mmHg to 121±1 mmHg. This blood pressure (121±1 mmHg) was significantly lower than that of control rats (134±2.7 mmHg).

Figure 2 shows the effects of the five fractions obtained by liquid liquid partition on rat blood pressure. 7-day-administration of E1 and E3 induced a significant reduction of rat MAP to respectively 128.5±6.4 mmHg and 134.2±4 mmHg, values which are similar to that of control rats. A

trend to a reduction of blood pressure was observed with extract E2, E4 and E5.

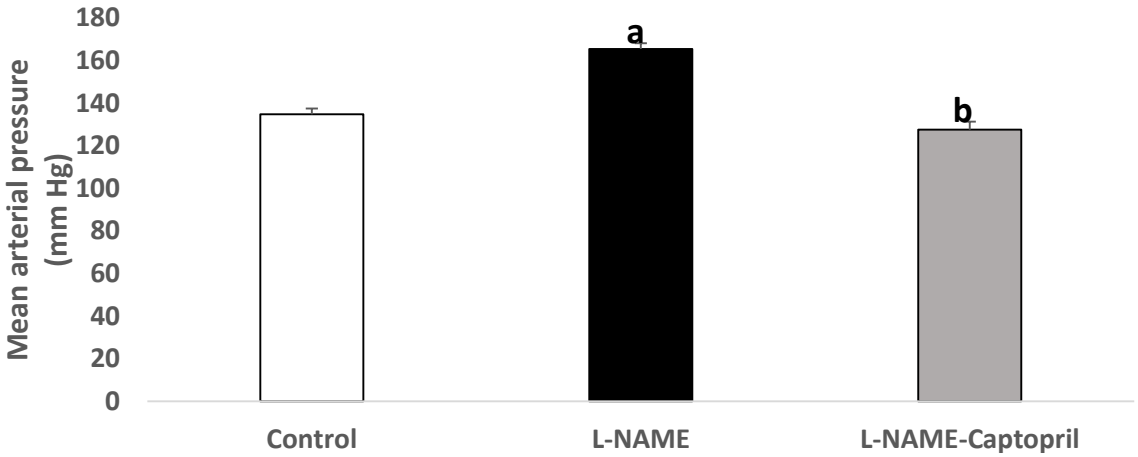


**Fig. 1:** Effect of L-NAME and captopril on wistar rat blood pressure.

L-NAME and L-NAME-Captopril, represent respectively groups of rats treated with respectively L-NAME and captopril following L-NAME administration.

Values of MAP (mean arterial pressure) are means ±SEM; n= 6/group

a: different from control value, $p<0.05$.

b: different from L-NAME group value, $p<0.05$.

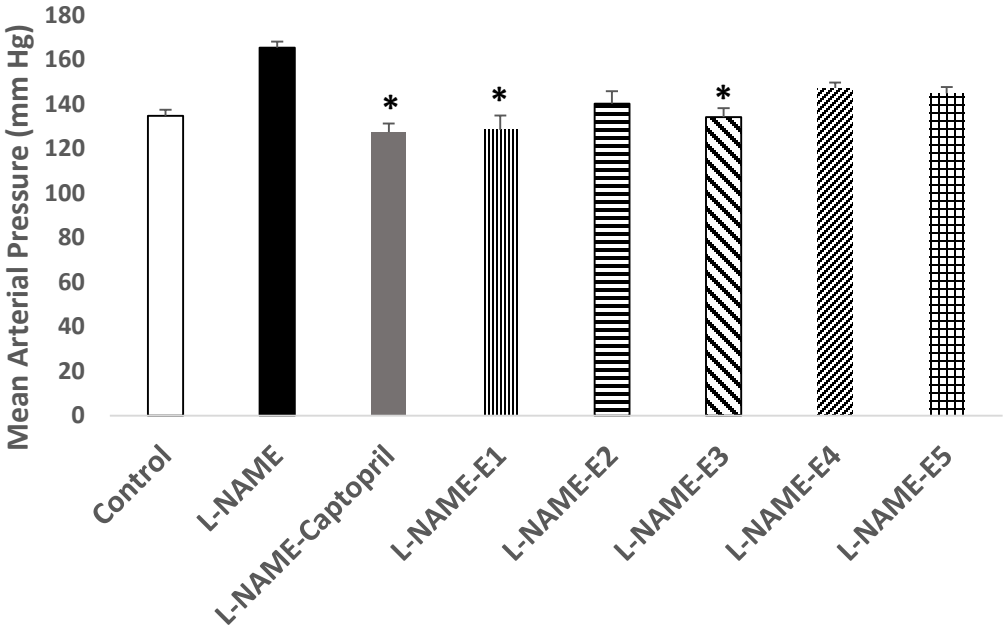


**Fig. 2:** Effect of *Gmelina arborea* fractions, obtained by liquid liquid extraction, on mean arterial pressure (MAP) of L-NAME-hypertensive rats.

L-NAME-Captopril, L-NAME-E1, L-NAME-E2, L-NAME-E3, L-NAME-E4 and L-NAME-E represent respectively groups of rats treated with respectively captopril, E1, E2, E3, E4, and E5 fractions after hypertension induction by N(G)-Nitro-L-Arginine-Methyl Ester (L-NAME) administration.

Values of MAP are means ±SEM; n= 6/group

*: different from L-NAME group value, $p<0.05$.

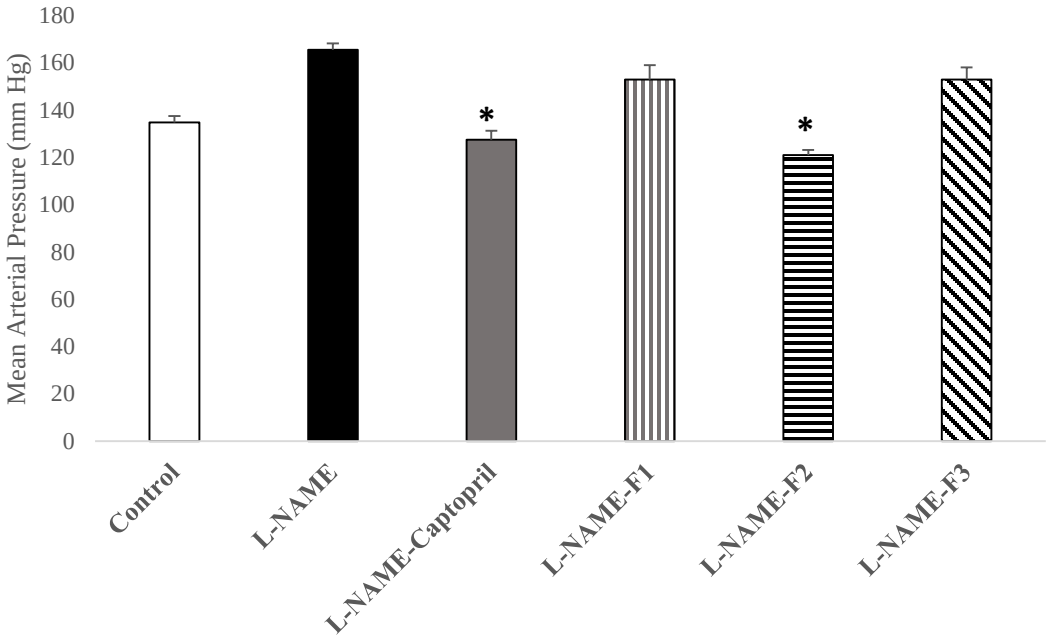


**Fig. 3:** Effect of *Gmelina arborea* fractions, obtained by sephadex gel chromatography, on mean arterial pressure (MAP) of L-NAME-hypertensive rats.

L-NAME-Captopril, L-NAME-F1, L-NAME-F2, L-NAME-F3, represent respectively groups of rats treated with respectively captopril, F1, F2, F3, fractions after hypertension induction by N(G)-Nitro-L-Arginine-Methyl Ester (L-NAME) administration.

Values of MAP are means ±SEM; n= 6/group

*: different from L-NAME group value, $p<0.05$.

## Discussion

In the present study, we showed that three of the eight fractions of *Gmelina arborea* crude aqueous extract induced a significant reduction of blood pressure in rats made hypertensive by L-NAME administration. This study is in agreement with our previous data showing an antihypertensive of the crude aqueous extract of *Gmelina arborea* (unpublished data).

Two chromatographic methods were used to separate the chemical compounds of the crude aqueous extract of *Gmelina arborea* leaves: the fractionation on Sephadex gel which separates compounds according to their molecular weight and the liquid-liquid partition which separates compounds according to their distribution index.

The phyto-chemical screening of the different fractions allowed us to characterize seven chemical groups. The detection of coumarins, flavonoids, saponins, anthracenes and tannins in some fractions (E2, E3, and F2) confirms data previously published (El Mahmoud *et al.,* 2010; Audipudi *et al.,* 2010). Likewise, flavonoids and coumarins detected in our fractions, have also been found in *Gmelina arborea* extract by Ngaman and co-workers (2009). However, naphthoquinones present in E1 extract was not detected in these previous studies.

Except F3 fraction, antioxidant activity has been observed with all the other fractions. As, free radicals contribute to more than one hundred of disorders in humans including the pathogenesis of hypertension and atherosclerosis (Vinay *et al.,* 2010), this antioxidant activity could be one of the mechanism involved in the antihypertensive effect observed.

The results obtained in the present study are in agreement with those of many studies (Vinay *et al.,* 2010; Ngaman *et al.,* 2009; Vijay *et al.,* 2011) Plants antioxidant activity is usually attributed to phenolic compounds (flavonoids, coumarins and tanins), compounds which are presents in most of the fractions. As for hypertensive activity, the most important activity was obtained with E1, E3 and F2

fractions. These fractions induced a significant reduction of rat blood pressure at the dose of 30 mg/Kg of body weight, dose which is 16.6 times lower than the active dose (500 mg/Kg) of the crude aqueous extract of Gmelina arborea leaves. These data suggest that the bioactive chemical compounds are actually concentrated in the active fractions.

Many chemical compounds detected in the active fractions could be involved in the antihypertensive activity. In E1 fraction, only naphthoquinones and flavonoids have been detected. Though naphthoquinones were not reported to have antihypertensive effects, flavonoids appear as the chemical compounds associated with the antihypertensive activity of this fraction. Flavonoids have been shown to induce vasorelaxation and to increase nitric oxide (NO) production by endothelial cells (Mendes *et al.,* 2011; Si *et al.,* 2014) although their vasoralaxation activity could involve NO independent mechanisms. In addition, the antihypertensive activity of quercetin, a flavonoid found in aerial parts of many plants including *Gmelina arborea*, has been demonstrated in many models of rat hypertension (Perez-Viscaino *et al.,* 2009).

Flavonoids was also detected in F2 and E3 where they were associated with alkaloids, anthracenes, tannins. In rat, many alkaloids have been shown to induce decreased heart rate and contractility and decreased vascular contractility (Mugabo *et al.,* 2012; Jadhar *et al.,* 2013). Antihypertensive activity associated with endothelium dependent vasorelaxant effect of tannins (proanthocyanidins) has also been described (Kawakami *et al.,* 2011; Furuuchi *et al.,* 2012).

## Conclusion

Three fractions, active on rat high blood pressure, have been obtained from the crude aqueous extract of *Gmelina arborea* leaves. In view of the chemical components detected in these fractions, flavonoids compounds appear as the most probable active chemical group although the antihypertensive activity observed could result from the synergic actions of many chemical groups. Further studies are necessary to isolate the active chemical groups and to investigate the mechanism of action on blood pressure.

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