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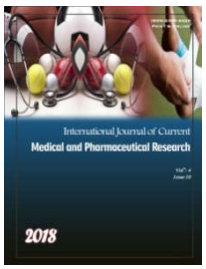


# CHEMICAL COMPOSITION, ANTIHYPERTENSIVE ACTIVITY AND IN VIVO TOXICITY OF LEAVES EXTRACTS OF TWO BENINESE MEDICINAL PLANTS

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**ABSTRACT**

The characterization of chemical constituents of alcohol-water extracts of *Parkia biglobosa* and *Spondias mombin* leaves revealed the presence of the following two important chemical groups: catechical tannins, reducing compounds and mucilage. Gallic tannins, flavonoids, leucoanthocyans, combined anthracenic derivatives (C-Heterosids), coumarin are found only in *Parkia biglobosa* leaves and saponosids are present only in *Spondias mombin* leaves. The alcohol-water extracts of *Parkia biglobosa* and *Spondias mombin* revealed *in vivo* an anti-hypertensive action respectively 171 mm Hg to 143 mm Hg, and 171 mm Hg to 154 mm on the rats whose blood pressure is higher than normal provoked by the L-NAME at the dose of 20mg/Kg/J. The anti-hypertensive effect of *Parkia biglobosa* crude extract is more important than the one of *Spondias mombin* crude extract. The study of acute toxicity of the two extracts carried out on NMRI mice allowed to classify these two species in the class III of World Health Organization (WHO): class of slightly toxic extracts with a lethal dose ($LD_{50}$ limit) higher than 2000 mg/Kg.



## INTRODUCTION

*Parkia biglobosa* or nere is a tree from 10 to 15m high with sunshade, alternate bipinnate leaves, and a characteristic tree of savanna in West Africa. It is a found on the Atlantic coast from Senegal till the south of Sudan and the north of Uganda. Traditional doctors use these plants in the treatment of much affection.

The leaves enter, by washing in the treatment of feverish states. The crushed leaves applied on the lips make disappear the spots of fever. We use the poultices and massage of crushed leaves for the external treatment of bronchitis. We prepare a lotion with the leaves and barks for eyeaches. The slightly grilled then crushed are applied on the wounds by burn. An infusion of the leaves or bark is used for the treatment of high blood pressure. The bark is rich in tannin. Infused, it gives a good herb tea to be taken after measles and smallpox.

The bark decoction vapoun relieves toothache and the decoction is used in gargle and mouth bath. The bark is used through external way as antifilarienne (Guinea worn), and by internal way as anti gonorrhoea with the *Cola cordifolia* and the *Khaya senegalensis*. The steeped bark decoction is used inbath: they also give it in drink against bilharzias (Adjanohoun *et al.*, 1989; Adjanohoun *et al.*, 2002).

*Spondias mombin* is a tree 10 to 25m high with alterned imparipennead leaves, condensed at the top of the branches. The flowers are white, small, very sweet smelling in 10 to 15 cm loose terminal panicle long and the fruits are egg- shaped

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and yellow when they are ripe and are 25 to 35 mm long, 15 to 20 mm wide with pleasant slightly acid pulp. It is a very widespread tree in the tropical countries because of its food virtues.

Its fruits recall a little the plum. The sweet astringent flesh and more or less slightly acid is pleasant but less abundant as far as the pit/stone is concerned. The cold juice can give a refreshing drink which is also used medically as febrifuge and diuretic. The fruit infusion is also used against fever. The pit contains prussic acid.

The almond is edible and is used against diarrhea. The very host vapors of the pits would relieve the pains of gout. The flowers are used in infusion against sore throat. The buds and the young branches produce a decoction for the treatment of eye related diseases. They also put these buds in hot bath, as astringents to strengthen the skin. They wash wounds and ulcers with leaves and buds decoctions. The leaves, in decoction were used as drink against dysentery. In infusion, they are a cure for cough. The leaves infusion is also used against fever and hypertension, whereas a decoction is a cure for gonorrhea. Fresh and young leaves juice is heated and pressed and given to children for the treatment of stomach disorders and a decoction of crushed and steeped leaves is used in lotion for eyes. In some regions the young leaves infusion is given to women giving birth to children in drink at the same time in hot astringent lotions. The leaves decoction is given in mouth bath as anti dontalgic. The plant is also used for the treatment of hemorrhage after childbirth. The bark contains a certain quantity of tannin. Boiled, it is used as mouth bath against toothache and in internal usage as worn tablets whereas in decoction it fights against violent coughs with inflammatory symptoms: it acts by bringing relief through vomiting effect. Dried and pulverized it is applied in bandage on circumcision wounds (Adjanohoun *et al.*, 1989; Adjanohoun *et al.*, 2002). The traditional therapeutic uses of these two plants mainly as far as hypertension is concerned made us anti-hypertensive action on the one hand and their toxicity on the other hand. The study whose results are shown is performed to search for a pharmacological support to the use of dried leaves ethanol-water extracts of these two plants.

## MATERIAL AND METHODS

### *Materials*

The leaves of *Parkia biglobosa* and *Spondia mombin* used traditionally for the treatment of high blood pressure were harvested and dried for ten days in a room at constant temperature (air conditioning) and carefully powdered with an electric grinder (*Flour MILLS NIGERIA, El MOTOR No 1827*). The male rats (middle weight 200g) which coming from the animalism of the teaching unit and Research in Human Biology of Health Sciences Faculty of Abomey-calavi University were used to measure arterial pressure while male mouse NMRI were used to evaluate acute toxicity.

### *Methods*

### *Phytochemical screening*

The phytochemical screening of plants was performed on their powder according to the standard procedures (Houghton and Raman, 1998; Houngbèmè *et al.,* 2014): Mayer's and Dragendorff's tests for alkaloids, Fehling's test for free reducing sugars and glycosides, Liebermann-Burchard's test for triterpenoids and steroids, frothy test for saponins, Shinoda's and sodium hydroxide tests for flavonoids, ferric chloride test for tannins, Guignard's test for free cyanogenetics derived and Borntrager's test for free anthraquinones.

### *Preparation of crude extracts*

100 g of each powdered plant were mixed with 500mL of water-alcohol (4/6, v/v). The mixture was macerated for 72 hours and filtered three times successively. Then the filtrate was evaporated to dryness at 40°C using a rotary evaporator (*Heidolph efficient Laborota 4000*) coupled to a water chiller (*Julabo FL 300*) to give the crude extracts (Houngbèmè *et al.*, 2014).

### *Antihypertensive assay*

This test was performed according to literature methods (Yang *et al.,* 2008; Paulis *et al.,* 2008; Lawson, 2009). The animals are weighed and anesthetized with thiopental at the dose of 40 mg/kg of corporal weight in intraperitoneal injection. Once anesthetized, the animal is placed on its back. The front legs are fixed along his body; the skin is plucked in the area of the neck and cleaned with alcohol 70 %. They perform one incision of the median plan of sternum upper part until 1cm from the lower jawbone. The sub-jawbone glands were opened with the help of tweezers, what permits to locate the trachea and withdraw delicately the carotid artery which a cord of vessels and nerves with the pneumogastric nerve. We carry out a ligation on the encephalic side of the carotid and we place a hemostatic sweezer on cardiac side to stop temporarily the blood. We incise the carotid and introduce the heparin catheter in the direction of the heart which is followed by the opening of the hemostatic tweezers by checking that there is no leak/escape. The catheter introduced is licked to the monitor who integrates the values of arterial pressure allowing us to read the figures of systolic arterial pressure. The plant extract was tested at the dose of 500 mg/Kg of corporal weight and per day. The L-NAME was administered at the dose of 20mg/Kg of corporal weight and during 7days. These different substances were administered by oral way with the help of stuffing probe. We formed 4 groups of 8 male rats.

- The 1st group received the L-NAME during 7days.
- The 2nd received the L-NAME during 7days followed by the administration of *Parkia biglobosa* crude extract during 7 days.
- The 3rd received the L-NAME during 7 days followed by the administration of *Spondia mombin* crude extract during 7 days.
- The 4th (witness one) received distilled water.

We took the arterial pressure with invasive measurement at the end of the 15th days for the 4 groups. We calculated the arterial pressure average (APA) for the pressure values obtained according to the following formula: APA = (2DAP+SAP) / 3; with DAP: diastolic arterial pressure and SAP: systolic arterial pressure.

### *Acute toxicity assay*

The animal groups for the oral $DL_{50}$ received respectively per groups: 250 mg/kg, 500 mg/kg, 750 mg/kg, 1500 mg/kg of corporal weight and the last group (the witness one) received distilled water for the oral way (esophagus probe stuffing). The groups of animals for the $DL_{50}$ by intra peritoneal way received respectively 125 mg/kg, 250 mg/kg, 500 mg/kg, 750 mg/kg, 1500 mg/kg, 2000 mg/kg and last one received distilled water (Ganfon *et al.,* 2012).

***Statistical analysis***

All data were expressed as mean +/- SEM. Student's t-test was used to determine significant differences between two groups. Mean values were considered significantly different when $p < 0,05$.

## RESULTS AND DISCUSSION

The results of the phytochemical analysis (Table 1) show that the gallic tannins, flavonoids, leuco-anthocyanins, C-glycosids, coumarins are found only in *Parkia biglobosa*. The saponins are presented only in *Spondias mombin*. The steroïds, triterpenes, alkaloids are only found in either plants.

The chemical compounds such as the gallic tannins, flavonoids found in majority in *Parkia biglobosa* would explain its high hypertensive activity to the *Spondias mombin* extract. In fact, the phenolic compounds mainly flavonoids and tannins are implied in the anti hypertensive activity by increasing the activity of endothelial NO-synthase and also by activation of the EDHF way of causing vasodilation of vessels by hyperpolarization of smooth muscle cells. (Ndiaye et *al.*, 2003; Ndiaye et *al.*, 2004).

The L-NAME administered at the dose of 20 mg/kg/j to the rats caused a significant rise of the medium arterial pressure value after 14 days (7 days of followed by 7 days of rest). The medium value of the arterial pressure obtained from the rats is 170 mm of Hg. We didn't notice side effects from the rats. The dose of 40mg/kg/j of L-NAME caused side effects such as: tearful eyes, hemorrhage and loss of urine which lead to the death of the animals.

The *Parkia biglobosa* crude extract administered at 500 mg/kg/day in hypertensive rats induced by L-NAME at 20 mg/kg/day resulted in a statistically significant decrease ($p< 0,05$) of the value of the mean arterial pressure after 14 days (7 days of treatment by L-NAME monitoring following by 7days of treatment by the extract). The mean value obtained is 140mm Hg (Table2).

The crude extract of *Spondias mombin* administered at 500 mg/kg/day in hypertensive rats induced by L-NAME at 20 mg/kg/day caused a relative decline in the value of the average blood pressure after of 14 days (7 days of treatment to L-NAME treatment following by 7days of treatment by the extract). The mean value obtained is 155 mm Hg This decline was smaller than that obtained for the crude extract of *Parkia biglobosa* (Table2).

The crude extract of *Parkia biglobosa* has an oral lethal dose ($LD_{50}$)limit > 2000 mg/kg body weight after 7 days which classifies it among the low toxicity extracts (OMS class III) with a very safety high index. Intraperitoneally we noted a mortality between 250 mg and 1500 mg/ kg body weight (Table3).

The $LD_{50}$ is 450 mg / kg body weight and its confidence limits are: $LD_{50}$ = 450 (328. 46; 616.5) mg/kg body weight or 328.46 mg/kg < 450 mg/kg < 616.5 mg/kg

The calculated variance $\mathbf{X^2_0}$ (0.3552) is much lower than $X^2_{0,05}$ (3.182) which ensures the validity of the experimental data.

The *Spondias mombin* crude extracts also has a limit oral $LD_{50}$ > 2000 mg/kg body weight after 7 days which also classifies it among the low toxicity extracts extracts (OMS class III) with an index of very high security. Intraperitoneally we observed mortality between 200mg and 3000 mg/kg of body weight (Table3). The $LD_{50}$ is 450 mg/kg body weight and its confidence limits are: $LD_{50}$ = 750 (551.47; 1020) mg / kg body weight or 551.47mg/kg < 750 mg/kg < 1020 mg/kg.
The calculated variance $\mathbf{X^2_0}$ (.070512) is significantly lower than $\mathbf{X^2}_{0.05}$ (2.776) which ensures the validity of the experimental data.

**Table 1** Results of Phytochemical Screening

| CHEMICALS GROUPS | *Parkia biglobosa* | *Spondias monbin* |
|---|---|---|
| ALCALOIDS | - | - |
| GALLIC TANINS | ++ | - |
| CATECHIC TANINS | ++ | + |
| FLAVONOÏD | + | - |
| ANTHOCYAN | - | - |
| LEUCOANTHOCYAN | + | - |
| QUINONIC DERIVATIVES | - | - |
| SAPONOSID | - | ++ |
| STEROÏD | - | - |
| TRITERPEN | - | - |
| MUCILAGE | + | + |
| REDUCE COMPOUNDS | + | ++ |
| CYANOGENIC DERIVATIVES | - | - |
| FREE ANTHRACENIC DERIVATIVES | - | - |
| COMBINED O-HETEROSID | - | - |
| ANTHRACENIC C-HETEROSID | + | - |
| COUMARIN | + | - |
| HETEROSIDES CARDIOTONIQUES | - | - |

- : absent or not reveled; +: present; ++: abundant.

**Table 2** Average of Arterial Pressure (PA) Calculated

| Compounds | average of PA (mmHg) ± standard error |
|---|---|
| control | 128,7 ± 1,5 |
| L-NAME | 171,5 ± 8,7 |
| L-NAME+*Parkia biglobosa* | 143,0 ± 11,5 |
| L-NAME+*Spondias mombin* | 154,1 ± 3,6 |

**Table 3** Results of Mortality Test to Oral $LD_{50}$ Determination for *Parkia biglobosa (Pb)* And *Spondias mombin (Spm)*

| Lots | Dose (mg/kg) | Number of rats | Number of death | | % of death | | Period (day) | |
|---|---|---|---|---|---|---|---|---|
| | | | *Pb* | *Spm* | *Pb* | *Spm* | *Pb* | *Spm* |
| Lot I | 250 | 6 | 0 | 0 | 0 | 0 | 7 | 7 |
| Lot II | 500 | 6 | 0 | 0 | 0 | 0 | | |
| Lot III | 750 | 6 | 0 | 0 | 0 | 0 | | |
| Lot IV | 1500 | 6 | 0 | 0 | 0 | 0 | | |
| Lot V | 2000 | 6 | 0 | 0 | 0 | 0 | | |
| control | | 6 | 0 | 0 | 0 | 0 | | |

*Pb: Parkia biglobosa ; Spm : Spondias mombin*

**Table 4** Results of Mortality Test of $LD_{50}$ Determination In Voice IP for *Parkia biglobosa* And *Spondias mombin*

| Lots | Dose (mg/kg) | Number of rats | Number of death | | % of death | | Period (day) | |
|---|---|---|---|---|---|---|---|---|
| | | | *Pb* | *Spm* | *Pb* | *Spm* | *Pb* | *Spm* |
| Lot I | 125 | 6 | 0 | 0 | 0 | 0 | | |
| Lot II | 250 | 6 | 2 | 2 | 16 ,66667 | 33,33334 | | |
| Lot III | 500 | 6 | 3 | 3 | 50 | 50 | | |
| Lot IV | 750 | 6 | 5 | 4 | 83,33334 | 66,66667 | 2 | 2 |
| Lot V | 1500 | 6 | 6 | 5 | 100 | 83,33334 | | |
| Lot VI | 2000 | 6 | 6 | 6 | 100 | 100 | | |
| Control | | 6 | 0 | 0 | 0 | 0 | | |

*Pb : Parkia biglobosa ; Spm : Spondias mombin*

## CONCLUSION

The phytochemical study revealed the presence of common way of the main chemical compound groups in the two studied

plant excerpts; these are the catechetic tannins, the reducing compounds and mucilages. Some chemical compounds are only found in the extract of *Parkia biglobosa*, it is about the gallic tannins, flavonoids, leucoanthocyans, and derivatives combined anthracen (C-Heterosids), the coumarins. On contrary, the saponosids found in the extract of *Spondias mombin*. The phenolic compounds found in the extract of *Parkia biglobosa* would be explain the origin of its high antihypertensive activity. The raw excerpt of *Parkia biglobosa* lowered significantly the value of the arterial hypertension provoked at the rats by the L-NAME. The diminution of the value of the arterial hypertension gotten with the extract of *Parkia biglobosa* is better in relation to the one gotten for the raw excerpt of *Spondias mombin*. The two raw extracts are very weakly active and belong in the III class of the classification of the OMS. For further investigation, the two crude extracts will be purified to isolate compounds which express anti-hypertensive activity.

**Competing Interests**

The authors declare that they have no competing interests.

**Authors' Contributions**

All the authors' participle in writing, giving feedback on this manuscript, have read and approved the final manuscript.

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