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Antihypertensive effect of extracts from *Crateva adansonii* DC.ssp. *adansonii* in the Wistar rats

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ABSTRACT

Crateva adansonii DC.ssp. *adansonii* (CA) is a medicinal plant used in traditional medicine to treat various diseases including hypertension. The main objective of this study was to assess the effect of the plant on high blood pressure. The crude aqueous extract of *CA* has undergone a liquid-liquid fractionation with increasing polarity solvents. The phytochemical analysis of the extracts was carried out by using the thin layer chromatography method. The pharmacological effect was evaluated in Wistar rats made hypertensive by administering the N (ω) -Nitro-L-Arginine-Methyl Ester (L-NAME). The crude extract was administered at 500 mg / kg (b/w) and the fractions (aqueous, butanol, dichloromethane and ethyl acetate) at 30 mg / kg (b/w). Blood pressure was measured by a non-invasive caudal arterial measurement method. Flavonoids, coumarins terpens, anthracens were detected diversely distributed either the crude extract or in the fractions. The crude aqueous extract induced a significant decrease in blood pressure from 155.6 ± 9.28 mm Hg to 106.00 ± 8.27 mm Hg. On the other hand, ethyl acetate and dichloromethane fractions exerted the highest effects among the fractions by reducing the blood pressure respectively from 139.8 ± 6.83 mmHg to 98.6 ± 8.38 mmHg and to 106.6 ± 6.80 mmHg. The results obtained justify the traditional use of the leaves of *CA* in the treatment of high blood pressure.

Keywords: *Crateva adansonii* DC.ssp. *adansonii*, medicinal plant, L-NAME, high blood pressure, Wistar rat, liquid-liquid chromatography.

INTRODUCTION

Cardiovascular diseases, dominated by high blood pressure, constitute a real public health problem in Africa. The correct management of arterial hypertension (ATH) is made difficult by the socio-economic context, unfavorable to developing countries and therefore inaccessible to a large stratum of the population. In order to respond their therapeutic needs; they generally use medicinal plants. *Crateva adansonii* DC. *ssp. adansonii* is one of the plants used to manage arterial hypertension in Benin (Tokoudagba et al., 2009).

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This plant has several therapeutic properties. In Republic of Benin, the plant is used in combination with other plants species by traditional healers and local populations. It is used as analgesic, antispasmodic, antimalarial and antidiarrheal (Agbankpè et al., 2015). The leaves are used as vegetables because of their great nutritional and therapeutic values (Dan Guimbo et al., 2012; Agbankpè et al., 2014; Kaboré et al., 2015). *Crateva adansonii* DC. ssp. *adansonii* is also used to treat mycotic diseases and also to accelerate the process of wound healing (Sabuj et al., 2008; Ajali et al., 2010). Populations use the leaves and bark of this tree to treat jaundice, eczema and rabies (Ganesan et al., 2009).

Many scientific studies have been carried out on the plant. The leaves extracts have anticancer, antimicrobial and antibacterial activities (Agboke et al., 2011; Lagnika et al., 2011; Zingue et al., 2016) and antiparasitic (Ngozichukwuka et al., 2012). The main objective of the present work was to evaluate the antihypertensive effects of this plant.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Crateva adansonii* DC. *Ssp.adansonii* were harvested in March 2015 in the town of Sèmè Podji, Division of Ouémé. Plant was identified and authenticated at the National Herbarium under the number AA 6481/HNB.The fresh leaves were washed with water, dried in a room maintained at temperatures between 16 °C for three weeks. The dried leaves were then pulverized using a grinder (MIKACHI-MK 1861 AP). The powder thus obtained was stored at room temperature for two weeks before use.

Extraction and fractionation

A mass of 100 g of dry powder of *Crateva adansonii* DC. *Ssp. adansonii* leaves and 750 mL of distilled water were introduced into a 1000 ml flask. The mixture was warmed up to 80 °C for 30 minutes using a heating cap. The decoction was filtered three times on cotton wool and then on WHATMAN No. 1 filter paper. The same process was repeated two (2) times on the residue. The watery extract obtained was concentrated under vacuum using a rotary evaporator of type Rotavapor Büchi R-3 (Sigma Aldrich, Germany) at 80 °C until a crude aqueous extract was obtained.

Thereafter, the crude aqueous extract was fractioned by successive exhaustion with increasing polarity solvents which includes cyclohexane, dichloromethane, ethyl acetate and butanol. Indeed, 50 g of the aqueous extract were dissolved with 500 ml of distilled water in a separating funnel. Then, 500 ml of cyclohexane was added to the solution; the whole was gently mixed and then left to stand for decanting. The organic phase was then recovered and the operation was repeated twice. The remaining aqueous phase was successively subjected to the same treatment as previously described with dichloromethane, ethyl acetate and butanol. The various fractions obtained were concentrated using an evaporator (Rotavapor). All the fractions were finally kept in an flask under a hood for 24 hours to evaporate any trace of solvent. These fractions were then stored in opaque vials at 4 °C.

Phytochemical analysis

The phytochemical analysis of the crude extract and the various fractions was carried out using a thin-layer chromatography (TLC) as described by Bladt and Wagner

(2001). 5 mg of each extract was dissolved in 1 ml of appropriate solvent (1: 1 methanol / water mixture, dichloromethane and ethyl acetate). On silica gel chromatography plates (60F254, Merck), 10 µl of each mixture were deposited and the migration was carried out using suitable solvents for each desired chemical group. Thus, for the searching of coumarins, flavonoids, tannins, triterpenes and anthocyanins derived, the migration solvent used was composed of ethyl acetate / formic acid / methanol / acetic acid / water (100: 11: 11: 26). The solvents used for the searching of alkaloids, anthracenes, and glucosides are a mixture of ethyl acetate, methanol and water (in proportions 100, 13.5: 10). For lignans, terpenes and sesquiterpenes, saponins, naphthoquinones, the migration solvents are respectively: chloroform / methanol / water (70: 30: 4); Chloroform / methanol / water (65: 25: 4); Chloroform / acetic acid / methanol / water (64: 32: 12: 8) and toluene/ formic acid (99: 1).

Experimental groups

Male Wistar strain rats weighing 190 ± 20 grams (3 months aged) were used. They were maintained in the animal room conditions (22 to 25 °C with a cycle of light / darkness oh 12 hours of Human Biology Unit of the Faculty of Health Sciences of Cotonou Benin. They have free access to tap water and food. The rats were made hypertensive conformely to the method described by Quenum et al (2014) with slight modifictions. Briefly, the rats were assigned to seven groups of five each. The control group which received only distilled water for 14 days. The second group which was administrated L-NAME at 40 mg / kg of body weight (b/w) from day 1 to day 7 and then distilled water from day 8 to day 14. The third group was treated by a reference antihypertensive drug (Losartan at 100 mg/kg b/w). And the last groups of rats treated with the four different extracts but after making them hypertensive from day 1 to day 7. All administrations were performed orally. The entire experimental procedure was carried out in compliance with institutional recommendations on ethics. The rats were kept under optimum conditions to minimize any form of suffering.

Measurement of blood pressure

Prior to the real experimentation, animals were trained to blood pressure measurement using the device for acclimation during 15 days. The arterial pressures of the rats were taken by non-invasive measurement with the CODA device (Kent Scientific Corporation, USA) on D0, D4, D8, D12 and D15. The CODA device allows the measurement of caudal arterial pressure in rats. This method of measurement consists in the use of a sleeve causing an occlusion of the blood flow at the level of the caudal artery. This sleeve is linked directly to a CODA voltage controller which is connected to a computer. Once the systolic and diastolic blood pressure values were obtained, the system automatically calculates the mean pressure while the cuff deflates completely. This value can also be calculated using the following formula: PAM = PAD + 1/3 (PAS-PAD).

Statistical analysis

Arterial pressure values are expressed as mean ± SEM. The data were entered in Excel and analyzed using GraphPad Prism 5 software. Analysis of Variance (ANOVA) followed by Bonferroni multiple comparison test was used for comparison between groups. Statistical significance was set at $p < 0.05$.

RESULTS

Aqueous extraction and liquid-liquid partition

The aqueous extraction carried out from 100 g of the powder of the leaves of *Crateva adansonii* DC. *ssp. adansonii* yielded 18.28 g of a dry extract of a slightly dark brown color with a yield of 18.28%.

The liquid-liquid partition from 50 g of crude aqueous extract allowed to obtain five (5) fractions: the aqueous fractions (Aqueous F.), cyclohexane (FC), dichloromethane (FDM), acetate Of ethyl (FAE) and butanol (F. BUTANOL). The masses, yields, colors and aspects of the various fractions are shown in Table 1.

The yields were determined based on the weight of the dry crude aqueous extract. The best yields were obtained with the aqueous phase followed respectively by the butanol phases, ethyl acetate, dichloromethane and cyclohexane. On the basis of very low yield obtained for the cyclohexane phase, we carried out a thin-layer chromatographic analysis of the various fractions in order to define their chromatographic profile with a view to possible regrouping. The reading of the plate revealed that the cyclohexane and dichloromethane fraction had the same profile and therefore consisted of the same families of chemical compounds. We have thus discarded the cyclohexane fraction (very low yield). Thus four (04) fractions were retained: the aqueous, butanol, ethyl acetate and dichloromethane fractions.

Phytochemical analysis

The summary of the results of the phytochemical screening is given in Table 2. Phytochemical analysis of the crude aqueous extract of *Crateva adansonii* DC. *ssp. adansonii* and different fractions revealed the presence of alkaloids in the fractions of ethyl acetate and dichloromethane, cardiac glycosides only in the ethyl acetate fraction, triterpenes and anthocyanin pigments in the crude extract and in all fractions, glycosylated terpenes in the dichloromethane fraction, glycosylated coumarins and tannins in the crude extract and in the butanol and ethyl acetate fractions, anthacenic derivatives in aqueous fractions and ethyl acetate ,bitter principles in the aqueous, butanolic and ethyl acetate fraction, glycosylated flavonoids, saponosides and lignans in the crude extract and the butanol, ethyl acetate and dichloromethane fractions. Saponosides in the crude extract and in the butanol and ethyl acetate fractions.

Effects of crude aqueous extract and fractions on arterial hypertension

Table 3 and Figure 1 showed the mean arterial pressures of the different batches of rats subjected to the study of the antihypertensive activity of the crude extract.

Administration of L-NAME from D1 to D7 resulted in a significant increase in rats PAM from 96.08 ± 6.97 mmHg (J0) to 155.6 ± 9.28 mmHg (J8) and 96.4 ± 11.80 mmHg (J0) at 159.2 ± 12.33 mmHg (J8) respectively for the positive control batch and the batch then treated with the crude extract.

Administration of the crude aqueous extract from D8 to D14 resulted in a significant decrease in rat PAM from 159.2 ± 12.33 mmHg (J8) to 106.00 ± 8.27 mmHg (D15).

Table 4 and Figure 2 showed the effect of the administration of the various fractions on the arterial pressure of the rats. Administration of L-NAME resulted in a significant increase of PAM. PAM normalized after administration of losartan, dichloromethane and ethyl acetate. This decrease was more marked with the ethyl acetate fraction than that with dichloromethane.

The butanol fraction induced a significant decrease but was not able to normalize the PAM of the rats whereas the aqueous fraction had no effect on the PAM of the rats.

Table 1: Summary of liquid-liquid partition efficiency results.

Fractions	Mass of fractions (g)	Extraction yield (%)	Colors and aspects after evaporation
F. AQUEOUS	22.09	44.18	Light brown and powder
FC	0.22	0.44	Powdery green and powder
FDM	0.54	1.08	Dark green pasty and powder
FAE	0.67	1.34	Paste and sticky yellow
FBUTANOL	3.13	6.26	Pasty and sticky brown
Total	26.35	53.3	

Table 2: Phytochemical screening of the crude extract and the different fractions of *Crateva adansonii* DC. *ssp. adansonii*.

Samples	Crude Extract	Aqueous fraction	Butanol fraction	Ethyl acetate fraction	Dichlromethane Fraction
Alkaloids	-	-	-	+	+
Glycosylated Coumarins	+	-	+	++	-
Glycosylated Flavonoids	++	-	+	++	+
Naphtoquinones aglycones	-	-	-	-	-
Anthocyanin pigments	+	+	++	+	+
Lignans	+	-	+	+	++
Saponosides	+	-	+	++	++
Anthracene Derivatives	-	+	-	++	-
Bitter Principles	-	+	+	++	-
Tannins	+	-	++	+	-
Glycosylated Terpenes	-	-	-	-	++
Triterpenes	+	+	+	+	++
Cardiac glycosides	-	-	-	+	-

Absence (-) (+) Low presence (++) Strong presence

Table 3: Comparative mean arterial pressure of the rats subjected to the study of the antihypertensive activity of the crude extract.

	D0	**D8**	**D15**
CONTROL	100,8 ± 8,25	97,6± 9,68	95,00 ± 9,35
L-NAME	96,8 ± 6,97	155,6± 9,28^{A***}	139,8 ± 6,83
L-NAME/CRUDE EXTRACT	96,4 ± 11,80	159,2± 12.33^{A***}	106,00± 8,27^{B***}

The data are expressed in mmHg and as mean +/- SEM
The values assigned to the letter A are significantly different from the value of the witness batch
The values assigned to the letter B are significantly different from the value of the L-NAME batch
*: P-value <0.05
****: p-value <0.001*

Table 4: Comparative mean arterial pressure of the rats subjected to the study of the antihypertensive activity of the different fractions.

	PAS	**PAD**	**PAM**
CONTROL	124,2 ± 4.20	79,8 ± 12.93	95,00 ± 9.35
L-NAME	165,00 ± 7.07	127,6 ± 7.36	**139,8 ± 6.83^{A**}**
L-NAME/Losartan	111,25 ± 7.32	76.75 ± 15.99	**88,00 ± 13.19^{B**}**
L-NAME/F AQ	158,8 ± 4.96	128,6 ± 7.33	133,2 ± 14.27
L-NAME/F BUT	148,2 ± 9.90	114,8 ± 9.20	**125,6 ± 9.23^{B*}**
L-NAME/F AE	119,4 ± 5.72	88,6 ± 10.66	**98,6 ± 8.38^{B**}**
L-NAME/F DM	129,00 ± 3.39	97,2 ± 7.46	**106,6 ± 6.80^{B**}**

F: Fraction; BUT: Butanol; DM: Dichloromethane; AE: Ethyl acetate.
The values assigned to the letter A are significantly different from the value of the control batch
The values assigned to the letter B are significantly different from the value of the L-NAME group
*: P-value<0.05
**: p-value <0.001

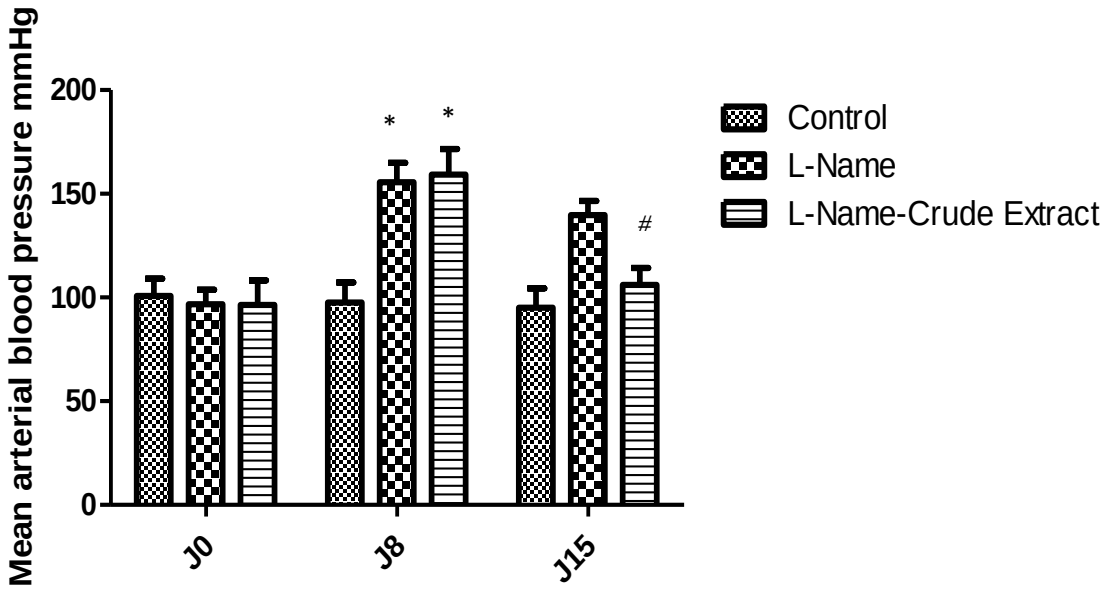

Figure 1: Effect of the crude aqueous extract of *Crateva adansonii* DC. *ssp. adansonii* on the arterial pressure of rats.

* Significant compared to controls group

\# Significant compared to L-NAME group

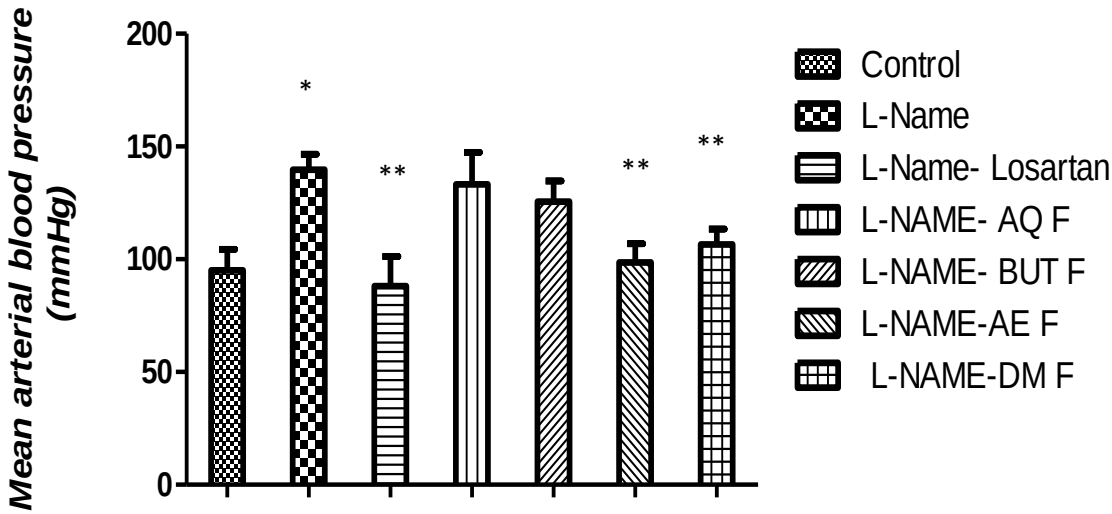

Figure 2: Mean arterial pressure of the different groups of rats.

** Significant compared to control group*

*** Significant compared to L-NAME group*

DISCUSSION

In Benin, aqueous decoction is the method used by healers to prepare herbal recipes. It is also the most widely used method for the preparation of recipes used in the traditional management of hypertension (Assogbadjo et al., 2005; Lucie et al., 2012). This technique has therefore been used to carry out the aqueous extraction of the plant drug which yielded 18.28%. Lagnika, in 2011 obtained a yield of 9.36% for the decoctate of the leaves of *Crateva adansonii* DC. *ssp. adansonii*. This difference could be explained by the fact that the residue was well depleted during extraction. Moreover, the harvesting sites, the season or the time of harvesting can influence the yield and the phytochemical properties of the extracts.

The liquid-liquid partition is carried out using solvents of increasing and appropriate polarity. Empirically, in the first intention, the aqueous extraction was chosen, followed by the alkaline extracts in conventional therapeutics. This choice is generally advisable because most active pharmacological molecules dissolve there. This method is similar to those used by Lukpata (2009) who carried out a fractionation of the methanolic extract of *Crateva adansonii* DC. *ssp. adansonii* using different organic solvents. The results of this partition showed that during the partition, a small quantity of extracts was carried into the organic phases, which justifies the better yield obtained for aqueous fraction (44.18%). In the organic phases, the butanol fraction gave the highest yield (6.26%) while the cyclohexane fraction gave the lowest yield with 0.44%.

The phytochemical screening allowed us to show the richness of the plant in twelve (12) chemical compounds distributed differently in the crude aqueous extract and the different fractions.
If alkaloids were found in the ethyl acetate and dichloromethane fractions in this study, Akanji et al. (2013) found them only in the aqueous extract of *Crateva adansonii* DC. *ssp. adansonii* leaves. Tsado et al. (2015), on the other hand, found these alkaloids only in the crude methanol extract of the leaves of *Crateva adansonii* DC. *ssp. adansonii*.
These differences may be related to geographic location, harvesting time of plant material, weather and storage conditions, plant physiological stage, or extraction solvent.

The glycosylated terpenes and the alkaloids absent in our aqueous extract were found in our dichloromethane and ethyl acetate fractions. The bitter principles, on the other hand, have been found in the aqueous, butanolic and ethyl acetate fractions. This could be explained by the fact that these compounds, in small amounts in the total aqueous extract and therefore not detectable, could be concentrated in the fractions.

The different fractions obtained and the crude aqueous extract were tested on a model of hypertension induced by L-NAME in the Wistar rat. Indeed, the inhibition of the synthesis of Nitrogen monoxide (NO) at the endothelial level induced by this substance is at the origin of the development of arterial hypertension in the rat. This inhibition of NO leads to vasoconstriction by increasing peripheral vascular resistance and is a relatively stable model of ATH study. According to Biancardi et al. (2007), vasoconstriction induced by sympathetic tone, in response to L-NAME administration, plays an important role in the initiation and maintenance of hypertension.

The dose of L-NAME at 40 mg / kg body weight used in our study is comparable to that used by Mali et al. (2012), and Bachav et al. (2012). Arterial pressures obtained after L-NAME administration were significantly higher than in normal untreated rats, indicating the efficacy of L-NAME at this dose. The aqueous extract studied significantly normalized the L-NAME-induced blood pressure at a dose of 500 mg / kg.

Measurements of arterial pressure reveal that the most important

antihypertensive activity was observed with the ethyl acetate fraction followed by that with dichloromethane. The butanol fraction also significantly reduces the PAM but does not standardize this pressure. On the other hand, the aqueous fraction induced no significant effect on the PAM. The effects of the ethyl acetate and dichloromethane fractions were obtained at a dose of 30 mg / kg body weight. This dose is 16.67 times lower than that of the total aqueous extract (500 mg / kg body weight) having given similar results. Thus, we can say that the partition was effective because it allowed to concentrate the molecules in the fractions. This would assume that the most active chemical families would be present in the three active fractions.

Our results were in line with those previously published (Adjagba et al., 2015) and were similar to those of Osseni et al. (2016) who, in their work, demonstrated that at a dose of 30 mg / kg body weight, the dichloromethane (DM) and ethyl acetate (AE) fractions of Tridax procumbens and Gmelina arborea significantly reduced PAM in the rat compared with the crude aqueous extract administered at 500 mg / kg body weight. Considering the model of ATH used during our study, the mechanism of action of the crude aqueous extract and the active fractions derived from the fresh leaves of *Crateva adansonii* DC. *ssp. adansonii* may be a relaxation of the vascular smooth musculature leading to a decrease in peripheral resistances due to the inhibition of the action of L-NAME.

The observed antihypertensive property could be related to the activity of the secondary metabolites detected in the aqueous extract and which were concentrated in the ethyl acetate fraction. The absence of bitter principles and anthracene derivatives in the crude aqueous extract but found in the active and non-active aqueous ethyl acetate fraction suggests that these two families of compounds were probably not involved in the antihypertensive effect observed. Similarly, glycosylated terpenes and *anthocyanin* pigments were present in all fractions and the crude aqueous extract and may not play a direct role in the observed effect but could have a synergistic action with the active molecules. Lupeol has been shown to have cardioprotective properties by protecting LDL (Low Density Lipoprotein) oxidation (Andrikopoulos et al., 2003). Lupeol and lupeol of acetate have also shown hypotensive activity, and could be considered as agents for the prevention of cardiac disorders and other cardiovascular diseases (Saleen et al., 2003). Studies have reported the presence of saponins, flavonoids, alkaloids (Abdullahi et al., 2012) of triterpenoids such as phragmatin triacetate, lupeol (Kplolali et al., 2010) and phenolic compounds in fresh leaves of *Crateva adansonii* DC. *ssp. adansonii* as possessing analgesic and anti-oxidant properties (Nkeiruka et al., 2015).

The antihypertensive activity of *Crateva adansonii* DC. *ssp.adansonii* would then be due to the synergistic action of flavonoids, coumarins, saponins and tannins. Indeed, saponins and flavonoids, due to their diuretic effect, could explain the decrease in the mean arterial pressure observed.

Molecules with high antioxidant potential such as rutin, quercetin, isoquercetin, which are flavonoids isolated from the leaves of *Crateva adansonii* DC. *ssp. adansonii*. Among these, Larson et al., (2010) reported that quercetin induced a significant decrease in arterial pressure of SHR rats, nitric oxide-deficient rats and rats pretreated with angiotensin II, but also an *in vitro* vasodilating action on the isolated rat aorta. The mechanisms involved would be a reduction in oxidative stress, inhibition of angiotensin converting enzyme activity or direct action on smooth vascular muscle.

Flavonoids represent a metabolic group which are a widely spread in plants. They also exhibit antioxidant activity and are involved in the prevention of cardiovascular diseases. Previous studies have demonstrated their

antihypertensive, vasorelaxant, vasoprotective effect. These compounds would contribute to the increased production of nitric oxide and prostacyclines by the endothelial cell.

The tannins would intervene in the inhibition of certain enzymes such as 5-lipo oxygenase and the angiotensin converting enzyme. These molecules could increase the bioavailability of nitrogen monoxide (NO) (Jian-Wei et al., 2009). These different chemical groups found in the extract would act synergistically to intervene in the regulation of cardiovascular disorders in the rat.

Conclusion

The use of a plant safely requires knowledge not only of its beneficial effects but also of the complications that can result from uncontrolled use. Our study examined the species *Crateva adansonii* DC. *ssp. adansonii*, a medicinal plant used in the treatment of arterial hypertension in Benin. It confirmed the antihypertensive activity of the crude aqueous extract of the leaves of this plant of the four fractions obtained by liquid-liquid partitioning of the crude aqueous extract, only the fractions with ethyl acetate and dichloromethane induced normalization of arterial pressure of the Wistar strain rats rendered hypertensive by administration of L - NAME. The most marked activity being obtained with the ethyl acetate fraction; Indicating the efficacy of the chemical compounds present in this fraction on arterial hypertension. All these results therefore constitute major arguments justifying the use of *Crateva adansonii* DC. *ssp. adansonii* in the treatment of ATH. The scientific basis of natural substances and the improvement of techniques by packaging in ready-to-use semi-finished form should enable the development of the improved traditional medicine.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. MA, RO, CH, GD designed the study, performed the literature searches and statistical analysis. BA, LL, RD and AL supervised this study and revised the first draft of the manuscript. All authors read and approved the final manuscript.

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REFERENCES

Adjagba M, Awede B, Nondichao K, Lagnika L, Osseni R, Darboux R, Laleye A. 2015. Antihypertensive Activity of Different Fractions of Tridax Procumbens Crude Aqueous Extract in Wistar Rats. *Journal of Physiology and Pharmacology Advances*, **5**(9): 713-719. DOI: https://10.5455/jppa.20150917122209.

Agbankpé AJ, Dougnon TV, Bankole HS, Yèhouénou B, Yedomonhan H, Legonou M, Dougnon TJ. 2014. Etude ethnobotanique des légumes feuilles thérapeutiques utilisés dans le traitement des diarrhées au sud-Bénin (Afrique de l'Ouest). *Int. J. Biol. Chem. Sci.,* **8**(4): 1784-1795. DOI: http://dx.doi.org/10.4314/ijbcs.v8i4.36.

Agbankpe AJ, Bankoles HL, Dougnon TJ, Yehouenou B, Hounmanou YMG, Baba-Moussa LS. 2015. Comparison of Nutritional Values of *Vernonia amygdalina*, *Crateva adansonii* and *Sesamum radiatum*: Three Main Vegetables Used in Traditional Medicine for the Treatment of Bacterial Diarrhoea

in Southern Benin (West Africa)**.** *Food and Public Health*, **5**(4): 144-149. DOI: http://dx.doi:10.5923/j.fph.20150504.07.

Agboke AA, Attama AA, Momoh MA. 2011. Evaluation of the antimicrobial properties of crude extract of *Cryptolepis sanguinolenta* and *Crateva adansonii* leaves and their interactions. *Journal of Applied Pharmaceutical Science,* **1**(10): 85-89. DOI: http://imsear.hellis.org/handle/123456789/151032.

Ajali U, Ezealisiji KM, Onuoha EO. 2010. Studies on wound healing properties of *Crateva religiosa* leaf extract. *Journal of Pharmaceutical and Allied Sciences*, 7(4). http://dx.doi.org/10.4314/jophas.v7i4.63457.

Akanji MA, Salau AK, Yakubu MT. 2013. Safety Evaluation of Aqueous Extract of *Crateva adansonii* Leaves on Selected Tissues of Rats. *Fountain Journal of Natural and Applied Sciences*, **2**(1): 17 - 28.

Andrikopoulos NK, Kaliora AC, Assimopoulou AN, Papapeorgiou VP. 2003. Biological activity of some naturally occurring resins, gums, and pigments against in vitro LDL oxidation. *Phytother Res*., **17**: 501-507. DOI: http://dx.doi.org/10.1002/ptr.118.

Assogbadjo AE, Caluwé ED, Sinsin B, Codjia J-CT, Damme PV. 2005. Indigenous knowledge of rural people and importance of baobab tree (*Adansonia digitata* L.) in Benin. Fourth International Congress of Ethnobotany (ICEB); 39-47.

Bachhav S, Bhutada MS, Patil SD, Baser B, Chaudhari KB. 2012**.** Effet of *Viscum articulatum* Burm. (Loranthaceae) in Nw-nitro-L-arginine methyl ester induced hypertention and renal dysfunction. *Journal of Ethnopharmacol.*, **142** (2):147-173. DOI: https://doi.org/10.1016/j.jep.2012.05.021 .

Dan Guimbo I, Barrage M, Douma S.2012. Etudes préliminaires sur l'utilisation alimentaire des plantes spontanées dans les zones périphériques du parc W du Niger. *Int. J. Biol. Chem. Sci*., **6**(6): 4007-4017. http://ajol.info/index.php/ijbcs

Djego J, Djego-Djossou S, Cakpo Y, Agnani P, Sinsin B. 2011. Evaluation du potentiel ethnobotanique des populations rurales au Sud et au Centre du Bénin. *Int. J. Biol. Chem. Sci*., **5**(4): 1432-1447. DOI: http://dx.doi.org/10.4314/ijbcs.v5i4.10

Ganesan S, Ponnuchamy M, Kesavan L, Selvaraj A. 2009. Floristic composition and practices on the selected sacred groves of Pallapatty village (Reserved forest), Tamil Nadu. *Indian Journal of Traditional Knowledge*, **8**(2):154- 162. http://www.niscair.res.in.

Igoli NP, Gray AI, Clements CJ, Igoli JO, Nzekwe U, Singla RK. 2012. Scientific Investigation of antitrypanosomal activity of *Crateva adansonii* DC leaves extracts. *Indo Global Journal of Pharmaceutical Sciences*, **2**(3): 226-229.

Jian-Wei G, Manning D, Young E, Shparago M, Sartin B, Bailey A. 2009. Vascular endothelial growth factor receptor inhibitor enhances dietary salt-induced hypertension in Sprague-Dawley rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol*., **297**(1): 142–148. DOI: http://dx.doi.org.10.1152/ajpregu.90972.2008.

Kaboré SA, Schumann K, Hien M, Lykke AM, Hahn K, Nacro HB. 2015 Stratégies d'adaptation à la réduction des services écosystémiques : cas des potentialités de substitution de trois espèces forestières dans le Sud-Ouest du Burkina Faso. *Int. J. Biol. Chem. Sci.,* **9**(3): 1194-1208. http://ajol.info/index.php/ijbcs

Kplolali YSA, Akossiwoa M-LQ, Agbonon I, Koumaglo K. 2010. Activités antioxydantes *in vitro* des feuilles de

Crataeva adansonii. Rev. Ivoir. Sci. Technol., **16**: 153 – 164.

Lagnika L, Anago E, Atindehou M, Adjahoutonon B, Dramane K and Sanni A. 2011. Antimicrobial activity of *Crataeva religiosa* Forst against bacteria isolated from Thryonomys swinderianus Temminck. *African Journal of Biotechnology*, **10**(49):10034-10039. DOI: http://dx.doi.org/10.5897/AJB10.2435.

Lucie AT, Dogo S, Valentin K, Emile AC, Mbacké S. 2012. Medicinal Plants Used In Some Rural Districts in Senegal (West Africa). *American-Eurasian Journal of Sustainable Agriculture*, **6**(4): 325-332.

Mali VR, Mohan V, Bodhankar SL. 2012. Antihypertensive and cardioprotective effets of the *Lagenaria siceraria* fruit in NG-nitro-L-argine methyl ester (L-NAME) induced hypertensive rats. *Journal of Pharmaceutical, Biological and Chemical Sciences,* **50** (11): 1428-35. DOI: http://dx.doi.org/10.3109/13880209.2012.684064.

Nkeiruka EU, Samuel OO. 2015. Analgesic and free radical scavenging activities of hydromethanolic extract of *Crateva adansonii* stem bark. *J Intercult Ethnopharmacol.,* **4**(3): 224–227. DOI: http://dx.doi.org/10.5455/jice.20150430010855.

Quenum CT, Ahissou H, Gouthon P, Laleye A. 2014. Etude de l'activité antihypertensive d'une association de plantes (*Schrankia leptocarpa, Garcinia kola* et *Ocimum americanum*) chez le rat Wistar. *Int. J. Biol. Chem. Sci.,* **8**(6): 2685-2695. DOI http://indexmedicus.afro.who.int

Sabuj S, Sagar KM, Prasana KP, Shyamlendu T, Satya RM, Poluri E, SASHI K, Dashsabuj S. 2008. Antimycotic potential of crataeva religiosa hook and forst against some selected fungal pathogens, *Acta Poloniae Pharmaceutica and Drug Research.*, **65**(2): 245-247.

Saleem R, Ahmad SI, Ahmed M, Faizi Z, Zikr-UR-Rehman S, Ali M, Faizi S. 2003. Hypotensive activity and toxicology of constituents from Bombax ceiba stem bark. *Biol Pharm Bull*., **26**(1):41-46. DOI: http://dx.doi.org/10.1248/bpb.26.41.

Tokoudagba J-M, Chabert P, Auger C, N'Gom S, Gbenou J, Moudachirou M, Schini-Kerth V, Lobstein A. 2009. Recherche de plantes à potentialités antihypertensives dans la biodiversité béninoise. *Ethnopharmacologia*, **44**: 32–41.

Tsado AN, Bashir L, Mohammed SS, Famous IO, Yahaya AM, Shu'aibu M Caleb T. 2015. Phytochemical composition and antimalarial activity of methanol leaf extract of *Crateva adansonii* in *Plasmodium berghei* infected mice. *British Biotechnology Journal*, **6**(4): 165-173.

Wagner H, Bladt S. 2001. *Plant Drug Analysis: a Thin Layer Chromatography Atlas* (2nd edn). Springer: Munich; 268.