Journal of Ethnopharmacology 132 (2010) 246–250

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Procyanidin-rich fractions from *Parkia biglobosa* (Mimosaceae) leaves cause redox-sensitive endothelium-dependent relaxation involving NO and EDHF in porcine coronary artery

Jean-Marie Tokoudagba[a,b,c], Cyril Auger[a], Lise Bréant[b], Saliou N'Gom[b], Philippe Chabert[b], Noureddine Idris-Khodja[a], Fernand Gbaguidi[c], Joachim Gbenou[c], Mansourou Moudachirou[c], Annelise Lobstein[b], Valérie B. Schini-Kerth[a,*]

[a] *UMR CNRS 7213, Laboratoire de Biophotonique et Pharmacologie, Université de Strasbourg, Faculté de Pharmacie, 67401 Illkirch, France*
[b] *UMR CNRS 7200, Laboratoire d'Innovation Thérapeutique, Equipe de Pharmacognosie et Molécules Naturelles Bioactives, Université de Strasbourg, Faculté de Pharmacie, 67401 Illkirch, France*
[c] *Laboratoire de Pharmacognosie et Huiles Essentielles, UFR Pharmacie, Faculté des Sciences de la Santé, Université d'Abomey-Calavi, 01 BP 188, Cotonou, Bénin*

ARTICLE INFO

Article history:
Received 28 May 2010
Received in revised form 12 July 2010
Accepted 6 August 2010
Available online 18 August 2010

Keywords:
Parkia biglobosa (Mimosaceae)
Polyphenols
Procyanidins
Vasorelaxant
Coronary artery
Endothelium

ABSTRACT

Aim of the study: Parkia biglobosa leaves are traditionally used as an antihypertensive agent in Benin. The present study assessed the vasorelaxant activity of different *Parkia biglobosa* leaf extracts using isolated porcine coronary artery rings.
Materials and methods: A hydroalcoholic leaf extract was submitted to a multi-step liquid–liquid fractionation with solvents of increasing polarity and the polyphenolic content of the different fractions was analyzed. Vascular reactivity of the different extracts was assessed using porcine coronary artery rings, in the presence or absence of specific pharmacological inhibitors.
Results: The hydroalcoholic, ethyl acetate and butanolic extracts contained mainly procyanidins and monomeric flavonoids. *Parkia biglobosa* leaf crude extract induced a redox-sensitive endothelium-dependent relaxation mediated by both nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). The fractionation of the butanolic extract generated 6 fractions, two of which induced stronger vasorelaxation than the original extract and they had a higher phenolic content.
Conclusions: Parkia biglobosa leaf extract is able to induce endothelium-dependent NO- and EDHF-mediated relaxation in porcine coronary artery rings. The vasorelaxant activity is dependent on their phenolic content and appears to involve mainly procyanidins.

1. Introduction

Hypertension is a major risk factor of cardiovascular diseases identified in western industrialized countries. However, the prevalence of hypertension and hypertension related cardiovascular diseases, such as ischemic heart diseases or stroke, is strongly increasing in developing countries and in particular in sub-Saharan Africa (Addo et al., 2007). Indeed, Mensah (2008) reported that while ischemic heart disease was recently considered as rare in sub-Saharan Africa, it could claim more than 600,000 lives in 2030. The demographic and nutritional transition in modern sub-Saharan Africa leads to the epidemiological shift toward cardiovascular diseases (Seedat and Rosenthal, 2006) and cancer as main causes of death. Moreover, treatments developed in industrialized countries for the control of blood pressure are not easily available to the population of sub-Saharan country, mainly due to high cost and limited accessibility of such drugs. Thus, a large number of patients refer to traditional healers for treatment of hypertension using selected local plants. Our previously published study reported the ethnopharmacological survey on the use of traditional plants for the treatment of hypertension in Benin (Tokoudagba et al., 2010), a sub-Saharan country with a high prevalence of cardiovascular risk factors in urban adults (Sodjinou et al., 2008). Among the studied plants, *Parkia biglobosa* leaves demonstrated the highest vasorelaxant activity in the porcine coronary artery (Tokoudagba et al., 2010). The genus *Parkia* belongs to the family Mimosaceae and compromises over 70 species. Only three of them are native of Africa: *Parkia biglobosa* Jacq. R. Br. ex G. Don, *Parkia bicolor*

Abbreviations: NO, Nitric oxide; EDHF, Endothelium-derived hyperpolarizing factor; L-NA, Nω-nitro-L-arginine; CTX, Charybdotoxin; APA, Apamin; SOD, Superoxide dismutase; GAE, Gallic acid equivalent.

* Corresponding author at: UMR CNRS 7213, Laboratoire de Biophotonique et Pharmacologie, Université de Strasbourg, Faculté de Pharmacie, 74, route du Rhin, BP 60024, 67401 Illkirch, France. Tel.: +33 368 854127; fax: +33 368 854313.
E-mail address: valerie.schini-kerth@unistra.fr (V.B. Schini-Kerth).

0378-8741/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2010.08.031

A. Chev., *Parkia filicoidae* Welw. ex Oliv. and one of Madagascar: *Parkia madagascaeriensis* R. Vig. (Burkill, 1995). *Parkia biglobosa* is a multipurpose fodder tree up to 20 m tall, with low branches, deciduous and flowering while leafless. It forms a wide-spreading crown planted as shade tree appreciated for reforestation. Its natural range extends in West African savannah land from the Atlantic Coast in Senegal to Sudan and Uganda. Many vernacular names are used: *néré* tree, *ahwa, ewé, igba, igiougba, ogba* illustrating its economic potential thought its various use by local populations (Hopkins, 1983). The fruit pods are dark brown and contain up to 30 seeds called African locust bean. The fermented seeds are well appreciated as a condiment in cooking under various names (e.g., *afitin* in Benin, *nététou* in Senegal, *dawadawa* in Nigeria) rich in proteins, sugars and vitamin B2 (Azokpota et al., 2006). All of the different parts of this plant are used by traditional healers for cure many disorders like hypertension, haemorrhages and dermatosis (Odetola et al., 2006; Udobi and Onaolapo, 2009). The use of stem bark is the most frequently cited in ethnological survey followed by the leaves of the plant prepared as a powder for a decoction (Gronhaug et al., 2008). Phytochemical studies have demonstrated the presence of known sterols and triterpenes from the petroleum extract of the bark. Long-chain *trans*- and *cis*-ferulate esters, lupeol, 4-*O*-methyl-epigallocatechin, epigallocatechin, epicatechin 3-*O*-gallate and epigallocatechin 3-*O*-gallate were isolated from the ethyl acetate extract of the bark (Tringali et al., 2000). The present study has assessed the biological activity of *Parkia biglobosa* leaf extract on the endothelial function, which plays a key role in the control of vascular tone predominantly via the release of potent vasodilators such as nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF), in blood vessels. In addition, experiments were also performed to characterize the active constituents underlying the vasorelaxant activity. We examined the vasorelaxant activity induced by a *Parkia biglobosa* leaf extract and several derived fractions in isolated rings of porcine coronary artery, and characterized the endothelium-derived vasorelaxant factor and the signaling pathway involved. In addition, using bioguided fractionation, we have characterized polyphenolic compounds present in the *Parkia biglobosa* leaf extract, which contribute to the vasorelaxant activity.

2. Materials and methods

2.1. Plant material and extraction

Fresh leaves of *Parkia biglobosa* were harvested in Bohicon on August 2006. The sample was authenticated by Pr Akoegninou at the National Herbarium, Cotonou, Benin where a voucher specimen (AA6330/HNB) was deposited. The sample was dried and ground into powder (100 g) before maceration under continuous stirring at room temperature with ethanol–water (6:4, v/v) (3× 300 ml, 72 h each). The filtered extracts were combined and evaporated under reduced pressure to obtain a dry extract (yield 21%).

2.2. Fractionation and isolation

Crude extract (20 g) suspended in water (100 ml) was successively and exhaustively extracted with cyclohexane (3× 200 ml), dichloromethane (3× 200 ml), ethyl acetate (3× 200 ml) and finally with n-butanol (3× 200 ml) giving 4 extracts (respective yields 1.25; 1.45; 17.90 and 17.00%) and a water residue (yield 46.20%). The butanolic extract (900 mg) was further fractionated on Sephadex LH-20 (Pharmacia, Sweden) column chromatography and eluted with the solvent systems comprising of water–methanol (100:0, 80:20, 60:40, 0:100, v/v) and then water–acetone (40:60, v/v) mixtures which gave 6 fractions in total called Fraction 1–6 (yields 26.80; 3.30; 13.78; 9.00; 28.31 and 12.49%, respectively). Each fraction was analyzed by thin-layer chromatography (TLC; Merck, Germany) eluted with ethyl acetate–formic acid–acetic acid–water (72:7:7:14, v/v/v/v) and revealed using Neu-PEG reagent at 366 nm. Spots were detected as flavonoids and phenolic acids by orange-yellow and blue fluorescence, respectively. Vanillin-sulfuric acid reagent was used for detection of catechins derivatives observed as red bands in daylight.

2.3. HPLC analysis

The extracts and all fractions were analyzed using an analytical RP-HPLC system comprising a Varian 9010 pump and a Varian ProStar® 330 diode array detector deuterium lamp (Varian, France) fitted with a column C18-Nucleodur®, 250 mm × 4.6 mm; 5 μm (Macherey-Nagel, Germany) and eluted with water (phase A) and methanol (phase B) using the following gradient: from 95 to 50% (A) for 10 min, from 50 to 30% (A) for 25 min and 100% (B) for 5 min and back to the initial conditions. The flow rate was 1 ml/min at 25 °C. The absorbance changes were recorded at 280 nm.

2.4. Determination of total phenolic contents

The total phenolic contents were determined in triplicate and expressed as mg gallic acid equivalents (GAE) using the Folin–Ciocalteu method (Singleton et al., 1999).

2.5. Vascular reactivity study

Pig hearts were collected from the local slaughterhouse. Left circumflex coronary arteries were excised, carefully cleaned of loose connective tissue and cut into rings (3–4 mm length). In some rings, the endothelium was removed mechanically by gently rubbing the lumen of the ring with forceps. Rings were suspended in organ baths containing oxygenated (95% O_2 and 5% CO_2) Krebs bicarbonate solution (mM: NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.18, $CaCl_2$ 1.25, $NaHCO_3$ 25, and D-glucose 11, pH 7.4, 37 °C) under a resting tension of 5 g for the determination of changes in isometric tension as described previously (Ndiaye et al., 2005). Rings were constricted with U46619, an agonist of thromboxan A2 receptor (9,11-dideoxy-9α,11α-methanoepoxy Prostaglandin $F_{2\alpha}$; Cayman Chemical, USA) to approximately 80% of the maximal contraction before a concentration–relaxation curve to an extract or fraction was constructed. In some experiments, rings were incubated with a pharmacological modulator for 30 min before addition of U46619.

2.6. 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 $P < 0.05$.

3. Results

3.1. Phytochemical analysis of the Parkia biglobosa extract and its fractions

The TLC and RP-HPLC analysis of the hydroalcoholic extract of *Parkia biglobosa* leaves revealed the presence predominantly of procyanidins and monomeric flavonoids. The content in total phenolic compounds was of 99.7 ± 3.2 mg of gallic acid equivalent (GAE) per gram of extract (Table 1).

Following the first fractionation using successive liquid–liquid extraction, the TLC analysis confirmed that the cyclohexane and dichloromethane extracts contained higher levels of apolar compounds and lower levels of phenolic compounds (15.9 ± 0.3 and

Table 1
Concentration of total phenols *in Parkia biglobosa* extracts and fractions measured by Folin–Ciocalteu assay.

	Total polyphenolic contents (mg GAE/g)
Extracts	
Crude extract	99.7 ± 3.2
Cyclohexane	15.9 ± 0.3
Di chl oromethane	68.9 ± 2.0
Ethyl acetate	303.7 ± 4.3
Butanol	212.7 ± 1.8
Aqueous	157.7 ± 2.8
Butanolic fractions	
F1	27.5 ± 0.6
F2	152.3 ± 0.6
F3	198.8 ± 1.7
F4	305.6 ± 13.9
F5	542.2 ± 3.3
F6	576.8 ± 11.7

Results are expressed as mg of gallic acid equivalent (GAE) per gram of extract under the form of mean ± SEM ($n = 3$).

68.9 ± 2.0 mg GAE/g, respectively). The TLC and RP-HPLC analysis indicated also that the ethyl acetate extract contained flavonoids and in particular catechins, whereas the butanolic extract contained ellagitannins, gallotannins, and procyanidins in addition to polar flavonoids. The ethyl acetate, n-butanol and aqueous extracts contained higher levels of phenolic compounds compared to the whole extract (303.7 ± 4.3, 212.7 ± 1.8 and 157.7 ± 2.8 vs. 99.7 ± 3.2 mg GAE/g, respectively).

The RP-HPLC analysis of the fractions obtained from the butanolic extract by gel chromatography on Sephadex LH-20 confirmed the presence of monomeric flavonoids and tannins. Fractions F1–F4 showed an increased concentration in monomeric flavonoids whereas fractions F5 and F6 contained mainly procyanidins and they had a higher total phenolic content (542.2 ± 3.3 and 576.8 ± 11.7 mg GAE/g, respectively).

3.2. Vasorelaxant effects

In porcine coronary artery rings, the *Parkia biglobosa* total extract induced concentration-dependent relaxations in rings with endothelium whereas only minor relaxations were observed in rings without endothelium (Fig. 1A).

In the presence of indomethacin to inhibit the formation of vasoactive prostanoids, the endothelium-dependent relaxations to the *Parkia biglobosa* total extract were minimally modified by either Nω-nitro-L-arginine, a competitive endothelial NO synthase inhibitor, or the combination of charybdotoxin and apamin, two specific inhibitors of EDHF-mediated relaxation (Fig. 1B). In contrast, the combination Nω-nitro-L-arginine plus charybdotoxin and apamin markedly reduced relaxations to the total extract indicating the involvement of both NO and EDHF (Fig. 1B).

Previous studies have indicated that the polyphenol-induced endothelium-dependent relaxation is a redox-sensitive event (Ndiaye et al., 2005; Madeira et al., 2009). Therefore, the role of ROS in the endothelium-dependent relaxation to *Parkia biglobosa* leaf extract was assessed. Relaxations were markedly reduced by the membrane-permeant analogue of SOD, MnTMPyP, and the membrane-permeant analogue of catalase, PEG-catalase (Fig. 2). In contrast, native superoxide dismutase and native catalase, which are unable to cross membranes, had no such effect (Fig. 2). These results indicate that endothelium-dependent relaxations induced by a *Parkia biglobosa* hydroalcoholic extract involves a redox-sensitive event requiring the intracellular formation of superoxide anions and hydrogen peroxide. To identify the phenolic compounds involved in the relaxation induced by the whole extract, several fractions were prepared and evaluated for their vasorelaxant activity. Compared to the total extract, only the butanolic and aqueous extracts showed similar vasorelaxant activity, while the ethyl acetate, dichloromethane and cyclohexane extracts were significantly less active (Fig. 3). The butanolic extract was further fractionated using Sephadex gel chromatography. Fractions F1–F4 were significantly less active whereas fractions F5 and F6 were significantly more active than the total and the butanolic extracts (Fig. 4).

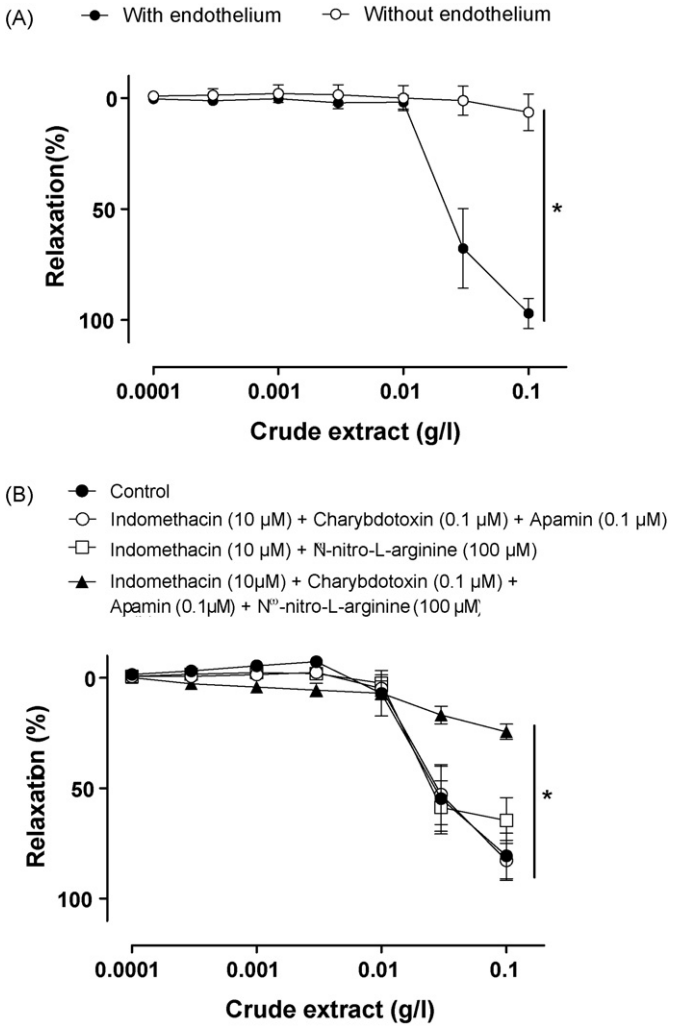

Fig. 1. Characterization of relaxations induced by *Parkia biglobosa* crude extract in porcine coronary artery rings (A). Intact and endothelium-denuded rings were contracted with U46619 before the addition of increasing concentrations of *Parkia biglobosa* extract. (B) Rings with endothelium were incubated with either Nω-nitro-L-arginine (L-NA, 100 μM, an inhibitor of endothelial NO synthase), charybdotoxin (CTX, 100 nM) plus apamin (APA, 100 nM; two inhibitors of EDHF-mediated relaxations) or the combination of L-NA, CTX and APA for 30 min before addition of U46619. All experiments were performed in the presence of indomethacin (10 μM) to prevent the formation of vasoactive prostanoids. Results are shown as mean ± SEM of five different experiments. *$P < 0.05$ vs. control.

4. Discussion

Previous investigations have indicated that a hydroalcoholic bark extract of *Parkia biglobosa* caused vasorelaxation (Kane et al., 2009), an aqueous bark extract decreased blood pressure in rabbits (Kassi et al., 2008), and a methanolic seed extract decreased blood pressure in the rat (Assane et al., 1993). The present investigations further extent these previous ones by showing that a leaf extract of *Parkia biglobosa* is a strong inducer of

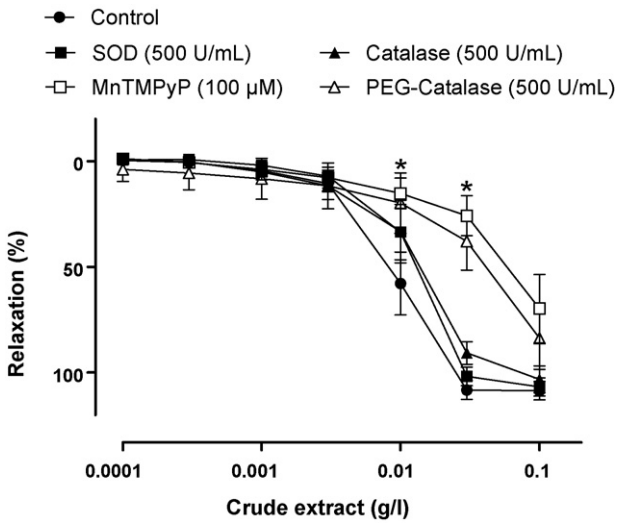

Fig. 2. *Parkia biglobosa* crude extract causes redox-sensitive endothelium-dependent relaxations in coronary artery rings. Rings with endothelium were exposed to either native superoxide dismutase (SOD), the membrane permeant SOD mimetic MnTMPyP, native catalase or the membrane-permeant catalase, PEG-catalase. Results are shown as mean ± SEM of five different experiments. $^{*}P < 0.05$ vs. control.

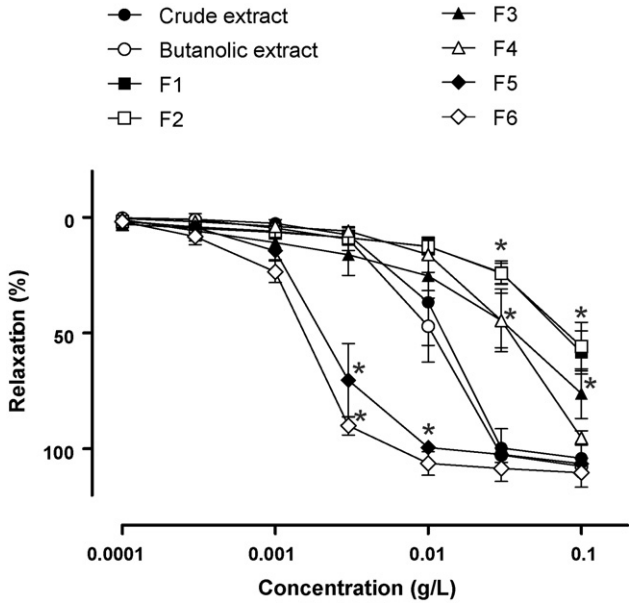

Fig. 4. Characterization of endothelium-dependent relaxations induced by different fractions of *Parkia biglobosa* in porcine coronary artery rings. Results are shown as mean ± SEM of five different experiments. $^{*}P < 0.05$ vs. control.

endothelium-dependent relaxations involving both NO and EDHF via a redox-sensitive mechanism. In addition, the fractionation of the leaf extract suggested that procyanidins are the major inducers of the vasorelaxation.

Most types of cardiovascular diseases including hypertension are associated with an endothelial dysfunction characterized by blunted endothelium-dependent relaxations. Thus, elevation of arterial blood pressure could be the consequence of an impaired formation and/or release of endothelium-derived relaxing factors NO and EDHF resulting in an exaggerated vascular tone. Therefore, the increase and/or restoration of the endothelial formation of relaxing factors by natural products is an interesting approach to normalize vascular tone and hence blood pressure (Schini-Kerth et al., 2010).

In the present study we have evaluated the potential of a *Parkia biglobosa* leaf extract to induce endothelium-dependent relaxations in porcine coronary artery rings. The data indicate that the hydroalcoholic leaf extract induced strong endothelium-dependent relaxations mediated predominantly by NO and EDHF. Moreover, the relaxations are reduced by membrane-permeant analogues of SOD and catalase but not affected by the corresponding native enzymes indicating that the induction of the relaxation involves a redox-sensitive event. These results are in agreement with previously published studies showing that polyphenol-rich sources (i.e., grape juice, red wine and a Crataegus extract) induced redox-sensitive NO- and EDHF-mediated endothelium-dependent relaxations (Auger et al., 2009). Moreover, it has been shown that the polyphenol-rich source-induced relaxation is mediated via the redox-sensitive activation of the Src/PI3-kinase/Akt pathway leading to endothelial NO synthase activation by phosporylation (Anselm et al., 2009).

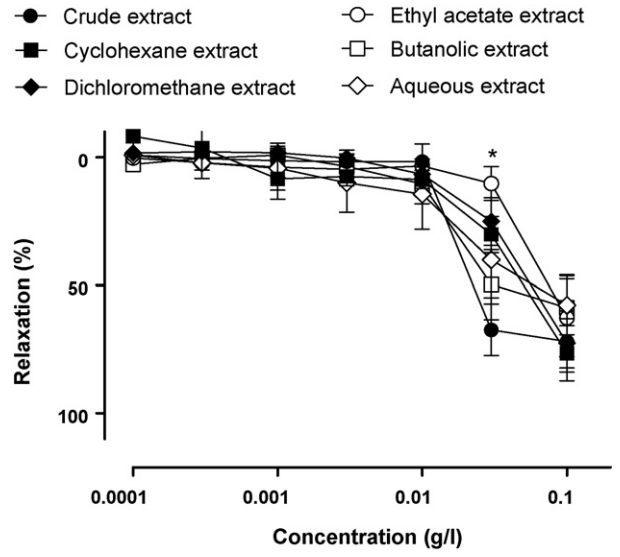

Fig. 3. Characterization of endothelium-dependent relaxations induced by different extracts of *Parkia biglobosa* in porcine coronary artery rings. Results are shown as mean ± SEM of five different experiments. $^{*}P < 0.05$ vs. control.

The fractionation of *Parkia biglobosa* leaf extract provided some indication on the nature of the compounds involved in the vasodilatation. The endothelium-dependent relaxation is predominantly observed with the butanolic and the aqueous extracts, which contain a mixture of monomeric flavonoids and procyanidins. The fractionation of the butanolic extract yielded six different fractions; two of these fractions induced stronger endothelium-dependent relaxations compared to the crude extract. The analysis of the different fractions indicated that the vasodilator activity is dependent on their phenolic content determined by the Folin–Ciocalteu method and on the presence of procyanidins.

Previous publications have indicated that several grape-derived products rich in polyphenols induce strong endothelium-dependent relaxations of various types of blood vessels (Andriambeloson et al., 1998; Soares de Moura et al., 2002). Moreover, Fitzpatrick et al. have shown that the procyanidin-type tannins are the major compounds involved in the relaxation induced by a grape-seed extract (Fitzpatrick et al., 2002). Additional studies have shown endothelium-dependent relaxations in response to extracts rich in procyanidins and gallotannins such as cocoa, maritime pine bark, apple and hawthorn (Karim et al., 2000). In addition, tannin-rich products have demonstrated blood pressure lowering effect in several experimental model of hypertension. Indeed, procyanidin-rich extracts from *Guazuma ulmifolia*

bark reduced blood pressure in spontaneously hypertensive rats (Magos et al., 2008). Similarly, a cocoa powder (up to 300 mg/kg body weight) reduced blood pressure as effectively as 50 mg/kg of captopril, an angiotensin converting enzyme inhibitor, in the spontaneously hypertensive rat (Cienfuegos-Jovellanos et al., 2009).

Finally, procyanidin-containing or flavonoid-rich products such as dark chocolates and cocoa beverages improved endothelial function in healthy subjects, hypercholesterolemic postmenopausal women, and in diabetic patients associated to an increased circulating level of NO species (Schmitt and Dirsch, 2009). Chronic intake of flavanol-rich dark chocolate also reduced blood pressure in patients with upper-range hypertension or stage 1 hypertension without concomitant risk factors (Grassi et al., 2005).

In conclusion, the present study reports the ability *of Parkia biglobosa* leaf extract to induce redox-sensitive endothelium-dependent relaxations in porcine coronary artery rings. The *Parkia biglobosa* crude extract-induced endothelium-dependent relaxation is mediated by both NO and EDHF, the two major endothelium-derived vasodilators. The fractionation of the leaf extract indicated that the vasorelaxant property is partially due to the presence of procyanidins. Altogether, these results indicate that *Parkia biglobosa* may exert beneficial effects on the endothelial function by decreasing vascular tone and hence support its traditional use in West African countries for high blood pressure treatment. In recent years, procyanidins have been well studied for their efficacy, low toxicity and high bioavailability. *Parkia biglobosa* is one of the wide-spread sources of procyanidins available for sub-Saharan populations and therefore constitutes one of the free accesses to heath care for hypertensive patients.

References

Addo, J., Smeeth, L., Leon, D.A., 2007. Hypertension in sub-Saharan Africa: a systematic review. Hypertension 50, 1012–1018.

Andriambeloson, E., Magnier, C., Haan-Archipoff, G., Lobstein, A., Anton, R., Beretz, A., Stoclet, J.C., Andriantsitohaina, R., 1998. Natural dietary polyphenolic compounds cause endothelium-dependent vasorelaxation in rat thoracic aorta. Journal of Nutrition 128, 1053–1058.

Anselm, E., Socorro, V.F., Dal-Ros, S., Schott, C., Bronner, C., Schini-Kerth, V.B., 2009. Crataegus special extract WS 1442 causes endothelium-dependent relaxation via a redox-sensitive Src- and Akt-dependent activation of endothelial NO synthase but not via activation of estrogen receptors. Journal of Cardiovascular Pharmacology 53, 253–260.

Assane, M., Baba Moussa, R., Bassene, E., Sere, A., 1993. Antihypertensive action of *Parkia biglobosa* (Jacq) Benth seeds in the rat. Dakar Médical 38, 49–54.

Auger, C., Chataigneau, T., Schini-Kerth, V.B., 2009. Vascular protection by grape-derived polyphenols. Agro Food Industry Hi-Tech 20, 38–40.

Azokpota, P., Hounhouigan, D.J., Nago, M.C., 2006. Microbiological and chemical changes during the fermentation of African locust bean (*Parkia biglobosa*) to produce afitin, iru and sonru, three traditional condiments produced in Benin. International Journal of Food Microbiology 107, 304–309.

Burkill, H.M., 1995. The useful plants of West Tropical Africa, vol. 3, 2nd edition. Families J-L. Royal Botanic Gardens, Kew.

Cienfuegos-Jovellanos, E., Quinones, M.M., Muguerza, B., Moulay, L., Miguel, M., Aleixandre, A., 2009. Antihypertensive effect of a polyphenol-rich cocoa powder industrially processed to preserve the original flavonoids of the cocoa beans. Journal of Agricultural and Food Chemistry 57, 6156–6162.

Fitzpatrick, D.F., Bing, B., Maggi, D.A., Fleming, R.C., O'Malley, R.M., 2002. Vasodilating procyanidins derived from grape seeds. Annals of the New York Academy of Sciences 957, 78–89.

Grassi, D., Necozione, S., Lippi, C., Croce, G., Valeri, L., Pasqualetti, P., Desideri, G., Blumberg, J.B., Ferri, C., 2005. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. Hypertension 46, 398–405.

Gronhaug, T.E., Glaeserud, S., Skogsrud, M., Ballo, N., Bah, S., Diallo, D., Paulsen, B.S., 2008. Ethnopharmacological survey of six medicinal plants from Mali, West Africa. Journal of Ethnobiology and Ethnomedicine 4, 26.

Hopkins, H.C., 1983. The taxonomy, reproductive biology and economic potential of *Parkia* (Leguminosae: Mimosoideae) in Africa and Madagascar. Botanical Journal of the Linnean Society 87, 135–167.

Kane, M.O., Sarr, M., Gueye, P.M., Ndiaye, C.A.B., Diaw, M., Ba, A., Sar, F.B., Sall Diallo, A., 2009. Vascular relaxant effects of a hydroalcoholic extract of bark of *Parkia biglobosa* (Mimosaceae). ScienceLib 2.

Karim, M., McCormick, K., Kappagoda, C.T., 2000. Effects of cocoa extracts on endothelium-dependent relaxation. Journal of Nutrition 130, 2105S–2108S.

Kassi, Y., Aka, K., Abo, K., Mea, A., Néné Bi, S., Ehilé, E., 2008. Effet antihypertensif d'un extrait aqueux d'écorce de tronc de *Parkia biglobosa* (Mimosaceae) sur la pression artérielle de lapin. Science Nature 5, 133–143.

Madeira, S.V., Auger, C., Anselm, E., Chataigneau, M., Chataigneau, T., Soares de Moura, R., Schini-Kerth, V.B., 2009. eNOS activation induced by a polyphenol-rich grape skin extract in porcine coronary arteries. Journal of Vascular Research 46, 406–416.

Magos, G.A., Mateos, J.C., Páez, E., Fernández, G., Lobato, C., Márquez, C., Enríquez, R.G., 2008. Hypotensive and vasorelaxant effects of the procyanidin fraction from *Guazuma ulmifolia* bark in normotensive and hypertensive rats. Journal of Ethnopharmacology 117, 58–68.

Mensah, G.A., 2008. Epidemiology of stroke and high blood pressure in Africa. Heart 94, 697–705.

Ndiaye, M., Chataigneau, M., Lobysheva, I., Chataigneau, T., Schini-Kerth, V.B., 2005. Red wine polyphenol-induced, endothelium-dependent NO-mediated relaxation is due to the redox-sensitive PI3-kinase/Akt-dependent phosphorylation of endothelial NO-synthase in the isolated porcine coronary artery. FASEB Journal 19, 455–457.

Odetola, A.A., Akinloye, O., Egunjobi, C., Adekunle, W.A., Ayoola, A.O., 2006. Possible antidiabetic and antihyperlipidaemic effect of fermented *Parkia biglobosa* (Jacq) extract in alloxan-induced diabetic rats. Clinical and Experimental Pharmacology and Physiology 33, 808–812.

Schini-Kerth, V.B., Auger, C., Kim, J.H., Etienne-Selloum, N., Chataigneau, T., 2010. Nutritional improvement of the endothelial control of vascular tone by polyphenols: role of NO and EDHF. Pflügers Archiv European Journal of Physiology 459, 853–862.

Schmitt, C.A., Dirsch, V.M., 2009. Modulation of endothelial nitric oxide by plant-derived products. Nitric oxide 21, 77–91.

Seedat, Y.K., Rosenthal, T., 2006. Meeting the challenges of hypertension in sub-Saharan Africa. Cardiovascular Journal of South Africa 17, 47–48.

Singleton, V.L., Orthifer, R., Lamuela-Raventos, R.M., 1999. Analysis of total phenols and other oxidation substrates and oxidants by means of Folin–Ciocalteu. Methods in Enzymology 299, 152–178.

Soares de Moura, R., Costa Viana, F.S., Souza, M.A., Kovary, K., Guedes, D.C., Oliveira, E.P., Rubenich, L.M., Carvalho, L.C., Oliveira, R.M., Tano, T., Gusmao Correia, M.L., 2002. Antihypertensive, vasodilator and antioxidant effects of a vinifera grape skin extract. Journal of Pharmacy and Pharmacology 54, 1515–1520.

Sodjinou, R, Agueh, V., Fayomi, B., Delisle, H., 2008. Obesity and cardio-metabolic risk factors in urban adults of Benin: relationship with socio-economic status, urbanisation, and lifestyle patterns. BMC Public Health 8, 84.

Tokoudagba, J.-M., Chabert, P., Auger, C., N'Gom, S., Gbenou, J., Moudachirou, M., Schini-Kerth, V., Lobstein, A., 2010. Recherche de plantes à potentialités antihypertensives dans la biodiversité béninoise. Ethnopharmacologia 44, 32–41.

Tringali, C., Spatafora, C., Longo, O.D., 2000. Bioactive constituents of the bark of *Parkia biglobosa*. Fitoterapia 71, 118–125.

Udobi, C.E., Onaolapo, J.A., 2009. Phytochemical analysis and antibacterial evaluation of leaf stem bark and root of the African locust bean (*Parkia biglobosa*). Journal of Medicinal Plants Research 3, 338–344.