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Original Article

Phytochemistry and Vasorelaxant activity of some plants used traditionnaly against high blood pressure in Benin

Jean-Marie Tokoudagba[1*]; Clément D. Gandonou[2]; Ursula Houngue[1] ; Cyril Auger[3] ; Valerie B. Schini-Kerth[3]

[1]Medicinal Organic Chemistry Laboratory School of Pharmacy, Faculté des Sciences de la Santé, Université d'Abomey-Calavi 01BP 188,Cotonou,Bénin
[2]Laboratoire d'Enzymologie et de Biochimie des Proteines, Faculté des Sciences et Techniques,Université d'Abomey-Calavi, 01BP: 188, Cotonou, Bénin
[3]UMR CNRS 7213, Laboratoire de Biophotonique et Pharmacologie, Université de Strasbourg, Faculté de Pharmacie, 67401 Illkirch, France

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ABSTRACT

The ethnobotanical survey conducted in Zou and Colline regions in February 2015 yielded twelve plants used traditionally against high blood pressure (hypertension) by the local population.. The phytochemical analysis and the biological screnning allowed to identify two plants more active (*Carissa eduliss* Apocynaceae, *Diodiascandess* Rubiaceae) than the active plant identified during a previous biological screnning carried out in 2006 [1]. These two active plants are very rich in phenolic compounds and have the same phytochemical profile in contrast to the others plants which have different phytochemical profile. The active plants have an antioxidant activity closer to that of the standards used. Some plants of the sample have a very strong antioxidant activity but are not active. The vasorelaxant activity of these two plants (*Carissa edulis* Apocynaceae, *Diodia scandess* Rubiaceae) is superior to the vasorelexant activity of *Parkia biglobosa* (Mimosaceae) [1]. Through this study, two new plants traditionally used against hypertension are retained among the twelve selected plants.

Keywords: Medicinal plants, arterial hypertension,vasorelaxant activity

Address for Correspondence: Jean – Marie Tokoudagba, Medicinal Organic Chemistry Laboratory School of Pharmacy, Faculté des Sciences de la Santé, Université d'Abomey – Calavi 01 BP 188 Cotonou Bénin,
E-mail: tokusj@yahoo.fr

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INTRODUCTION

High blood pressure is a pathology affecting both northern and southern countries [2, 3]. It is both a chronic disease and a risk factor since it exposes to serious complications and pathologies including stroke. In 90% of cases, the cause of hypertension is unknown, so multiple modifiable lifestyle factors must be considered; to the environment and non-modifiable related to age; heredity and certain diseases or medicinal treatment (kidney or endocrine diseases, antidepressants, corticosteroids) [4].

The majority of African population only uses plants that surround them for treatment. Several medicinal plants known as antihypertensive are used alone or in combination and in various forms by local populations. The question is whether these plants really have these virtues. The traditional therapeutic uses of these plants especially with regard to hypertension led us to evaluate their antihypertensive activity though an ethnobotanic survey. Then, the phytochemical mechanisms related to the antihypertensive activity of selected plants were studied.

METHODS AND MATERIAL

Inventory of plants with antihypertensive reputations: We used bibliographic databases, specialized works, publications and supplemented these data by field surveys in the medicinal plant markets of these selected regions in the presence of guides who knew both the regional languages and plants. We also surveyed ten traditional practitioners exercising in private clients and registered in the national directory of Traditional Medicine promotion of Public Health Ministry. During the survey the questions asked are relative to

- the plants used for hypertension
- the vernacular names of the plants used
- the part of the plant used
- the method of preparation for the dosage
- the mode of administration of the recipes

Twelve species of plants were selected among the most widely used and least studied species based on phytochemical and pharmacochemical aspects.

Plant material and extraction: Fresh leaves of these plants were harvested in Abomey and Dassa on February 2015. The sample was authenticated by Pr Akoegninou at the National Herbarium, Cotonou, Benin where a voucher specimen 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 heach). The filtered extracts were combined and evaporated under reduced pressure to obtain a dry extract (yield 21%).

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 (3–4mmlength). 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-glucose11, pH 7.4, 37 ◦C) under a resting tension of 5 g for the determination of changes in isometric tension as described previously [5]. 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.

Phytochemical screening: The phytochemical screening of the extracts was performed according to the standard procedures: Mayer's and Dragendorff's tests for alkaloids, Fehling's test for free reducing sugars, Fehling's test for 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

Determination of polyphenolic compounds

Total polyphenols: The total phenolic content of the various extracts was quantified using the Folin–Ciocalteu reagent according to Singleton et al.[6]. This method consist to use a mixture of phosphotungstic and phosphomolybdic acids which was reduced during the oxidation of phenols into a mixture of tungsten blue oxide and molybdenum [7]. The absorbance was measured by a spectrophotometer (JENWAY 50/60 Hz) to 765 nm. Gallic acid was used as reference and the total polyphenol content in the extract was expressed by mg of Gallic acid equivalent per gram of dry matter.

Total Flavonoids: The method of aluminum trichloride ($AlCl_3$) was used to quantify the total flavonoids. This technique was based on the formation of the aluminum complex flavonoids that has a maximum absorption at 500 nm [8-9].

Condensed tannins: The condensed tannins dosing was achieved by the method of sulfuric vanillin [10, 11]. The principle of this assay was based on the binding of vanillin aldehyd group on the carbon in position 6 of the ring of the catechol to form a red colored complex chromophore which absorbed at 510 nm.

Evaluation of scavenging activity: The scavenging activity was evaluated by the DPPH method [12]. The principle of this method was based on measuring the trapping free radicals in a solution of DPPH. This trapping wasindicated by the disappearance of the purple color of DPPH. The mixture of DPPH solution and the sample was left in the darkness for an hour and the absorbances measured at 517 nm. The trapping percentage was determined by the formula: P= $(Ab_W-Ab_S)/Ab)$ X100; P: percentage of trapping; Ab_W: absorbance of the white; Ab_S: Absorbance of the sample.

RESULTS AND DISCUSSION

Twelve plants have been selected from the medicinal plants used as antihypertensive treatment in the pharmacopoeia and traditional medicine in Benin through the ethnobotanical survey of plants recorded in the regions of Zou and Colline of Benin.

Phytochemical analysis of the extracts of these plants revealed the strong presence of catechin and gallic tannins in some plant species, especially in plants active at vasorelaxant test. These plants are: *Parkia biglobosa* (Mimosaceae), *Tridax procubens* (Asteraceae) and *Fucus exasperata* (Moraceae). However, only catechin tannins are presents in *Paulina pinnata* (Sapindaceae) and only gallic tannins in *Trema guineensis* (Ulmaceae).

Flavonoids are only present in active plants which are Carissa edulis (Apocynaceae) Diodia scandess (Rubiaceae) and Parkia biglobosa (Mimosaceae). Saponosides and free anthracenics are found in some plants species. The reducing compounds and steroids are found in the majority of plants. Other families of compounds such as O-heterosides, C-heterosides, cardiotonic derivatives, cyanogenic derivatives, quinonics, coumarins and alkaloids are not present in most of the selected plant species.

The determination of phenolic compounds in plant species showed a high content of gallic acid equivalent (total polyphenols), quercetin equivalent (flavonoids) and catechin equivalent (condensed tannins) in five including the active plants at the vasorelaxant test.

The antioxidant activity related to the radical activity of the active plants is not so high compared to the activity of the standards. Whereas other plants have shown strong antioxidant activity and are not active (*Tridax procubens, Fucus exasperate* et *Trema guineensis*). We can say that the antioxidant activity of the plants is not linked to the vasorelaxant activity.

The study of vasorelaxant activity revealed tree plants of which two are more active (*Carissa edulis* et *Diodia scandess*) than the third one (*Parkia biglobosa*)[1]. These two more active plants have the same phytochemical profile at the opposite of the bird plant (*Parkiabiglobosa)* which contain coumarins and leucoanthocyanins in his phytochemical profile.

Since phenolic compounds are involved in vasorelexant activity, we could deduce that the high content of phenolic compounds is at the origin of the vasorelaxant activity observed in these plants.

CONCLUSION

This study allowed us to retain two new plants traditionally used in hypertension among the twelve selected plants. The results thus obtained offer a contribution to the valorization of beninese traditional medicine which imposes the implementation of scientific procedures.

TABLE 1: PHYTOCHIMIC CARACTERISATION OF THE TWLEVE PLANTS

Composés	1-*Trema guineensis*	2-*Paulina pinnata*	3-*Biophytum petersianum*	4-*Tridax pocubens*	5-*Fucus exasperata*	6-*Lantana camara*	7-*Parkia biglobosa*	8-*Spondias mombin*	9-*Brysocarpus coccineus*	10-*Eleusine coracana*	11-*Carissa eduliss*	12-*Diodias scandens*
Catéchiques	-	++	-	++	++	-	++	+	-	-	++	++
Tanins Galiques	++	-	-	++	++	++	++	-	-	-	++	+++
Flavonoïdes	-	-	-	-	-	-	+	-	-	-	+	++
Anthocyanes	-	-	-	-	-	-	-	-	-	-	-	-
Leuco-anthocyanes	-	-	++	-	-	-	+	-	-	-	-	-
Saponosides	-	++	-	-	-	+	-	++	++	++	++	++
Dérivé cyanogéniques	-	-	-	-	-	-	-	-	-	-	-	-
Triterpènes	-	-	-	-	-	++	-	-	-	-	-	-
Stéroïdes	++	++	++	++	++	-	-	-	++	++	++	++
Alcaloïdes	-	-	-	-	-	+	-	-	-	-	-	-
Coumarines	-	-	-	-	-	-	+	-	-	-	-	-
Composés Réducteurs	++	++	++	-	++	+	+	++	++	++	+	+
Quinoniques	-	-	-	-	-	-	-	-	-	-	-	-
Mucilages	-	++	-	-	-	-	+	+	+	+	-	-
Anthraceniques libres	-	-	-	-	++	-	-	-	-	++	++	++
O-hétérosides	-	-	-	-	-	-	-	-	-	-	-	-
C- hétérosides	-	-	-	-	-	-	+	-	-	-	-	-
Dérivés cardiotoniques	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 2: CONCENTRATION OF POLYPHENOLS, FLAVONOID TOTAL, AND OF CONDENSED TANINS AND ANTIRADICAL ACTIVITEES

	Dosage des composés phénoliques						Activités antiradicalaires		
	PT (AGE)		FLA (QE)		Tac (CE)		DPPH (IC_{50})	Standards	
Fucus exasperata	36,80	0,81	33,89	0,44	158,41	4,62	0,36	AG	0,03
Tridax procubens	12,27	0,16	12,07	0,13	49,30	4,29	0,56	BHA	0,09
Carissa eduliss	80,03	1,01	391,76	3,42	443,61	6,62	0,03	Q	0,1
Biophytum petersianum	99,89	1,29	208,53	2,28	193,92	0,65	0,036		
Lantana camara	54,88	0,98	65,75	1,71	57,27	2,49	0,12		
Eleusine coracana	76,34	0,87	318,31	2,80	963,85	16,76	0,135		
Spondia mombin	171,83	1,81	389,62	6,84	316,82	1,32	0,014		
Paulina pinnata	22,29	0,10	48,22	0,46	71,18	1,90	0,2		
Diodia scandens	79,67	1,38	260,02	4,15	267,32	0,59	0,015		
Brysocarpus coccineus	58,80	0,30	57,72	0,43	54,015	3,08	0,09		
Trema guineensis	27,02	0,21	18,78	0,36	43,10	1,46	0,38		
Parkia biglobosa	86,49	1,32	117,52	0,28	248,13	0,66	0,09		

Q : Quercetine ; AG : Acide Gallique ; BHA : ButhylHydroxylAnisol ; DPPH : 1,1-DiPhényl-2-PicrylHydrazyle

TABLE 3 : LIST OF PLANTS SELECTIONED

Espèces	Famille
Ficus exasperata	Moraceae
Tridax procubens	Asteraceae
Carissa eduliss	Apocynaceae
Biophytum pertersianum	Connaraceae
Lantana camara	Verbenaceae
Eleusine coracani	Poaceae
Spon dia mombin	Anacardiaceae
Paullinia pinnata	Sapindaceae
Diodia scandens	Rubinaceae
Brysocarpus coccineus Schum et Thonn	Connaraceae
Trema guineensis	Ulmaceae
Parkia biglobosa	Mimosaceae

DROITES DE CALIBRATION POUR LE DOSAGE DES COMPOSES PHENOLIQUES

FIGURE 1 : DOSAGE DES POLYPHENOLS TOTAUX (ACIDE GALLIQUE EQUIVALENT)

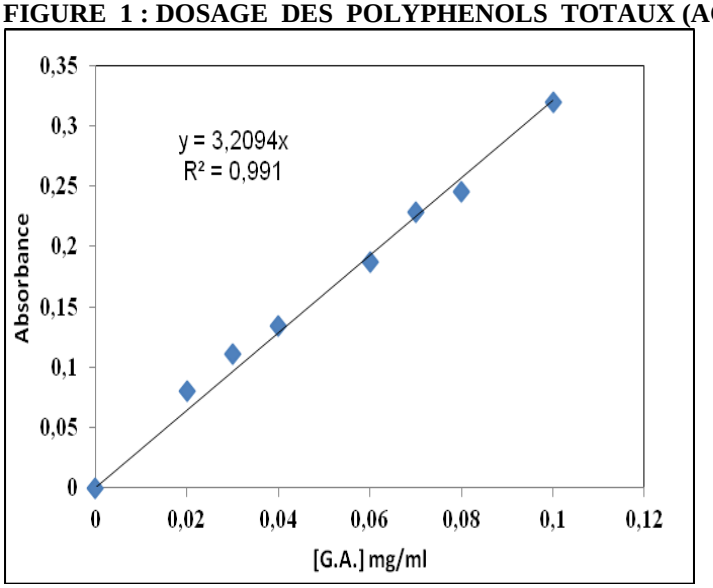


FIGURE 2 : DOSAGE DES FLAVONOIDES TOTAUX (QUERCETINE EQUIVALENT)

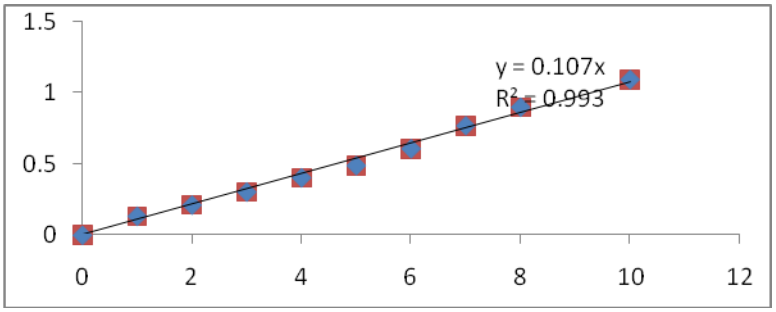


FIGURE 3 : DOSAGE DES TANINS CATECHIQUES (CATECHINE EQUIVALENT)

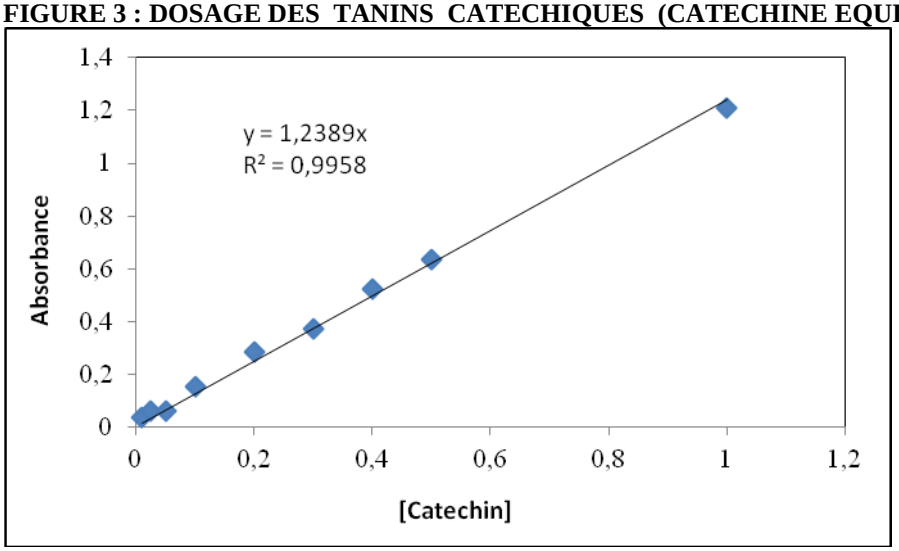


FIGURE 4 ; COURBES VASORELAXANTS DES PLANTES NON - ACTIVES

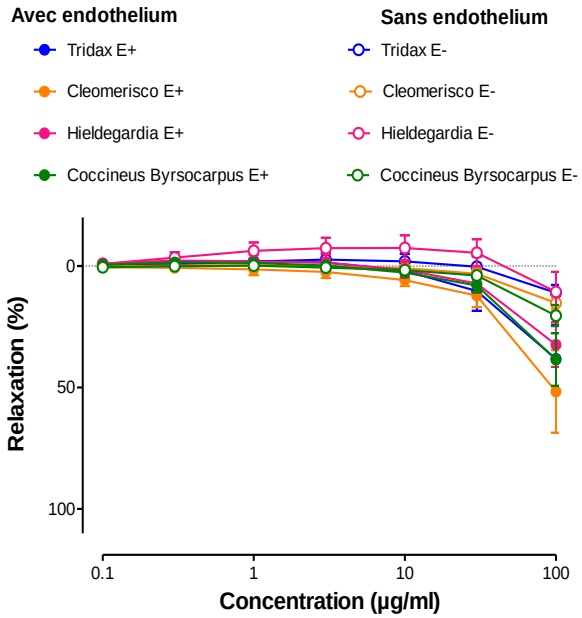


FIGURE 5 : COURBES VASORELAXANTES DES PLANTES MONTRANT L'ACTIVITE DE *PARKIA BIGLOBOSA*

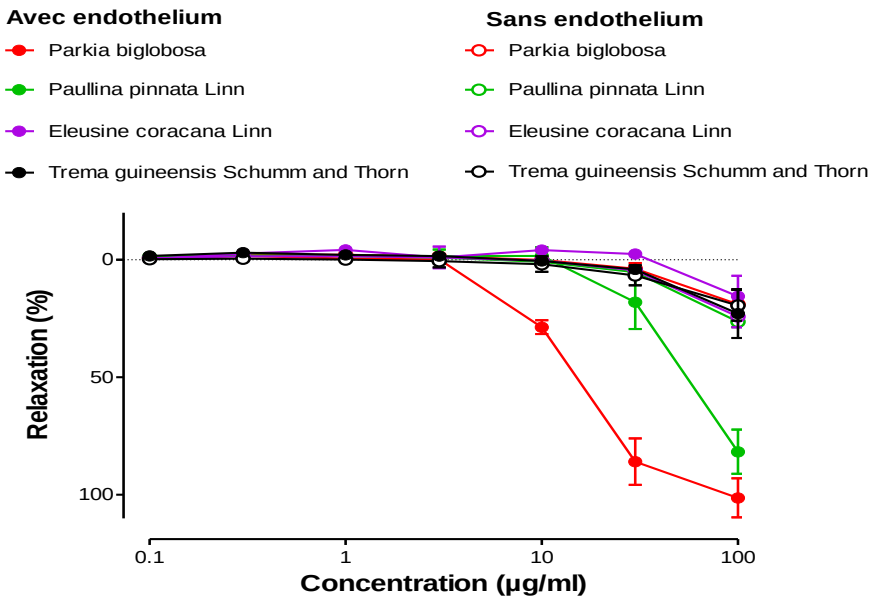
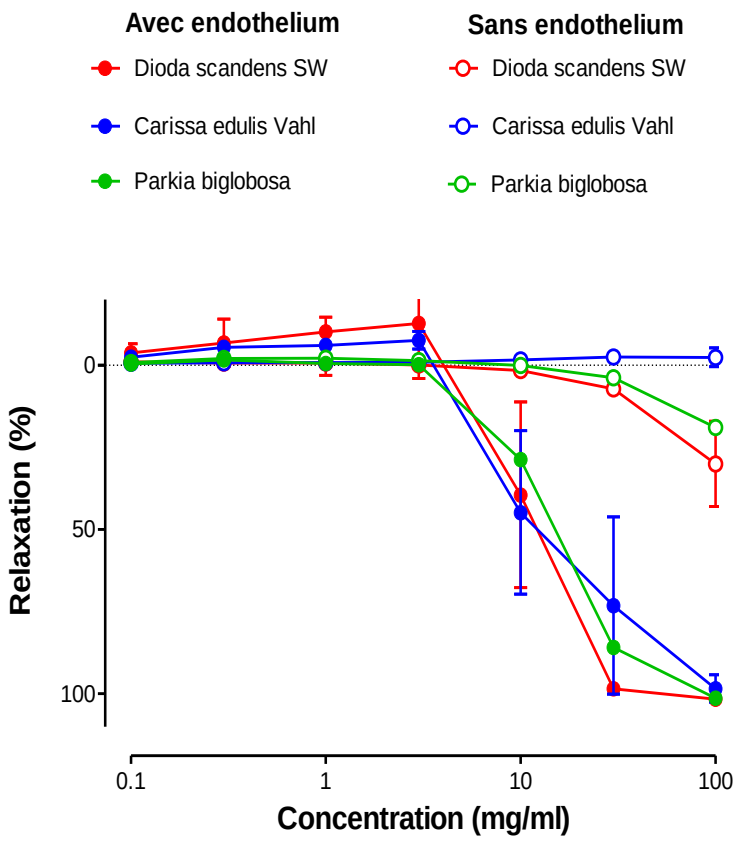


FIGURE 6 : COURBES VASORELAXANTES MONTRANT L'ACTIVITE DES PLANTES ACTIVES



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