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## STUDY OF MEDICINAL PLANTS USED IN THE MANAGEMENT OF CARDIOVASCULAR DISEASES AT LIBREVILLE (GABON): AN ETHNOPHARMACOLOGICAL APPROACH

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### ABSTRACT

#### Keywords:

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*Guibourtia tessmannii*,  
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This work was conducted at a Libreville herbal market located in Peyrie in order to inventory plants used by people for the management of cardiovascular diseases such as hypertension and to evaluate their pharmacological effects. The method of preparation and modes of administration were also recorded. Twenty nine herbalists were interviewed using questionnaires. Twenty two plant species belonging to sixteen families and seventeen recipes were identified. The commonly used plants were *Guibourtia tessmannii*, *Musanga ceropioiodes*, *Senecio gabonensis*. Among them, *G. tessmannii* appeared to be the most used plant species. Phytochemical studies on extracts of *G. tessmannii* revealed the presence of alkaloids, sugars, polyphenols, sterols, tannins and saponosids. Pharmacological studies performed in the isolated aorta of rats showed a vasorelaxant effect on adrenalin- or KCl- induced contraction. *G. Tessmannii*-induced vasorelaxation was significantly but not totally reduced by endothelium removal or by a pretreatment with L-NAME, suggesting the involvement of endothelium-dependent and -independent mechanisms. Medicinal plants and *G. tessmannii* in particular may represent a source of efficient antihypertensive agents.

**INTRODUCTION:** Hypertension was for a long time considered as a disease affecting only people in developed countries. However, nowadays, it represents a public health problem for developing countries in Africa, especially in Gabon. According to WHO estimations, in 2000, 26.4 % of the world population (26.6 % male and 26.1 % female) was affected by hypertension and developing countries represent the most affected part of the world.

Indeed, two third (2/3) of the 972 million adults suffering from hypertension in the world are living in these countries. The prevalence of this affliction is expected to increase by 29.2 % (29 % are men and 29.5 % women) by 2025. Cardiologists in Gabon predict that hypertension could become the first cause of mortality by 2020. According to the same specialists, there is a warrant need to develop screening, control and preventive methods to fight hypertension<sup>1</sup>.

Gabon has, like many developing countries; a population which increasingly uses herbal medicine to manage hypertension. At the same time, at the Institute of Pharmacopoeia and Traditional Medicine (IPHAMETRA) in Gabon, a large program of research on medicinal plants is carried out in order to contribute effectively to the management of various diseases including hypertension via development of phytomedicines in accordance with government guidelines. The program involves ethnobotanical surveys, experimental laboratory research and the production of herbal medicines. There are a large number of documented plants used for the management of hypertension<sup>2,3,4</sup>. Plant remedies are often used by people by auto-medication<sup>5,3</sup>.

Furthermore, few medicinal plants remedies have been scientifically validated for the treatment of cardiovascular diseases since the majority of them have not been experimentally evaluated. The present study aimed to identify medicinal plants and remedies used by healers to treat cardiovascular diseases such as hypertension in Libreville, the capital of Gabon, where nearly half of the population lives. We aimed to validate plants or remedies with efficient therapeutic value leading to the production of accessible, effective and economically affordable phytomedicines.

## MATERIAL AND METHODS:

**Study Area:** The ethnobotanical investigation was conducted in Mont-Bouet market, the largest market in Gabon, located at Libreville (0°23'24"N 9°27'15"E), the capital city. It is the most populated town of Gabon with a cosmopolite population estimated at 684,800 people. Libreville is located in north-western Gabon, in the Estuaire Province.

**Plant Material:** Experiments were performed on *Guibourtia tessmannii*, a genus of trees containing 17 species which are found in tropical Africa and tropical America. *Guibortia tessmannii* is one of the largest trees occurring in primary terra firma forests in Cameroon and Gabon and found from 30-280 meters above sea level. The stem bark of *G. tessmannii* was collected from the Monts de Cristal, in the Estuaire province (Gabon) during the rainy season. All plant materials in this study were identified by R. Niangadouma, botanist of the National Herbarium

where a voucher specimen of *G tessmannii* (SRFG 879 LBV) was deposited.

**Animals:** Animals used in this study were guinea-pigs (*Cavia porcellus*) weighing 180-300 g. Animals were fed with grass and were acclimated to the environment before experiments began.

**Chemicals:** Acetylcholine (ACh), Adrenalin (Ad), L-NAME, were purchased from Sigma Aldrich Company (St. Louis, Missouri, USA). Petroleum ether, methanol, acid chlorhydric (HCl), sulfuric acid were sourced from Merck (France).

**Ethnobotanical Survey:** Ethnobotanical data were obtained from 29 herbalists using a semi-structured survey.

The following information was obtained:

- Ethnicity and age of the herbalists;
- Vernacular names of the plants sold;
- Part of plants used in the preparation;
- Parts of the plant used;

**Methods of drug preparation, administration and dosage of remedies:** Plant materials were collected and were authenticated by an expert botanist at National Herbarium, a department of the Institute of Pharmacopoeia and Traditional Medicine (IPHAM ETRA), referring to Herbarium specimens and the books on Gabon flora.

**Preparation of the Methanol Extract of the plant:** Air-dried stem barks of *Guibourtia tessmannii* were crushed into small pieces using a Culatti micro-crusher. About 250 g of fine powder was macerated in 200 ml of methanol during 24 hours. Then, the aqueous extract was filtered using a Whatman Millipore filter. The solvent obtained was evaporated using a Bucchi rotavapor (Heidolph, type: Laborota 4000) and the sample of 9.5 g of powder (the methanolic extract) was conserved at +5°C.

**Phytochemical Analysis:** The characterization of chemical groups such as sterols, alkaloids, tannins, polyphenols, reductor compounds and saponosides was performed using the methods of Sofowora<sup>6</sup> and Harborne<sup>7</sup>.

**Recording of the Contractile Activity of the Guinea-pig isolated aorta:** Animals were killed by cervical dislocation. The aorta was dissected, cleaned of connective tissue and cut into approximately 5-6 mm strips. Aorta rings were placed in petri-dish containing the Mac-Ewen solution with the following composition (mM): NaCl, 130; KCl, 5.6 CaCl<sub>2</sub>, 2.6; NaH<sub>2</sub>PO<sub>4</sub>, 0.91; NaCO<sub>3</sub>H, 11.9, MgCl<sub>2</sub>, 0.24; glucose, 11. Experiments were performed on the intact aorta and on endothelium-denuded vessels. The endothelium was removed by gently rubbing the interior of the vessel with a disposable cotton applicator.

Aortic ring was mounted in an organ bath containing the Mac Ewen solution oxygenated with carbogen (95% O<sub>2</sub>, 5% CO<sub>2</sub>) and maintained at 37°C using a LAUDA Ecoline 003 circulating bath (Hugo Sashes Electronic, March-Hugstetten, Germany).

One end of the aortic strip was attached to a metal hook and the other one was connected to an isometric force transducer (F30 HSE 372, Hugo Sachs Electronic, March-Hugstetten, Germany). Isometric tension was amplified using an amplifier (D-79232 March, Hugo Sachs Electronic, Germany) and was recorded on a Rikadenki multi-pen recorder (R-6 4-D, RIKADENKI CO., LTD, Japan)

Aortic ring was equilibrated during 90 min. under a resting tension of 1g. After equilibration, tissue viability was confirmed by adding KCl (80 mM) or Ad (2.5x10<sup>-2</sup> mg/ml).

Endothelium integrity responsiveness was verified through relaxation of Ad-induced contraction with ACh (10<sup>-6</sup> mg/ml). The functional removal was verified by the absence of relaxation evoked by ACh on Ad-induced contraction. The Aortic strip was then washed with Mac-Ewen solution to allow its relaxation to the lowest tension.

To evaluate the EMGT-induced vasorelaxation, cumulative doses of EMGT were added to the organ bath, to induce relaxation after a submaximal contraction of the aortic vessel by KCl or adrenalin. The endothelium-denuded strip was treated with the L-

NAME (inhibitor NO-synthetase) 15 min before pre-contraction with adrenalin followed by the addition of the cumulative concentrations of the plant extract to the bath.

**Statistical Analysis:** All values are expressed as means ± S.E.M. Multiple comparisons were performed with a one-way ANOVA followed by Dunett's test to determine the difference between the group means. Value was considered Significant for p <0.05.

**RESULTS:** The investigations performed at Mont-Bouet herbal market were conducted on a sample of 29 herbalists, from whom 11 agreed to collaborate with us (10 females and 1 male). Several traditional healers didn't want to participate to the ethnobotanical survey in order to protect their knowledge from scientists. Some of them wanted to be paid or wanted to sale their remedies with the recipes we needed.

Our study allowed us to inventory 17 medicinal recipes used for the management of hypertension. These recipes were developed from 22 plant species belonging to 16 families (**Table 1**). About 59% of the recipes were mono-specific, whereas 41% of them were combinations of plant species.

The most cited plant species were *G. Tessmannii* (29.41%) followed by *Picralima nitida*, *Persea cratissima* and *Senecio gabonensis* (23.26% for each species). The parts of plants used, the methods of preparation and administration are recorded in **Table 2**. Leaves were the most-used parts (40.9%) followed by stem bark (36.36%), fruits (13.63%), liana and clove of garlic (13.36%).

Decoctions represent the most utilized mode of preparation (88.23%). The administration routes of all the recipes were oral.

Phytochemical analysis of the extracts of *Guibourtia tessmannii*'s stem bark revealed the presence of the following compounds: sterols, alkaloids, tannins, polyphenols, sugars and saponosides (Table 2).

TABLE 1: DATA OF MEDICINAL PLANTS USED FOR THE MANAGEMENT OF CARDIOVASCULAR DISEASES

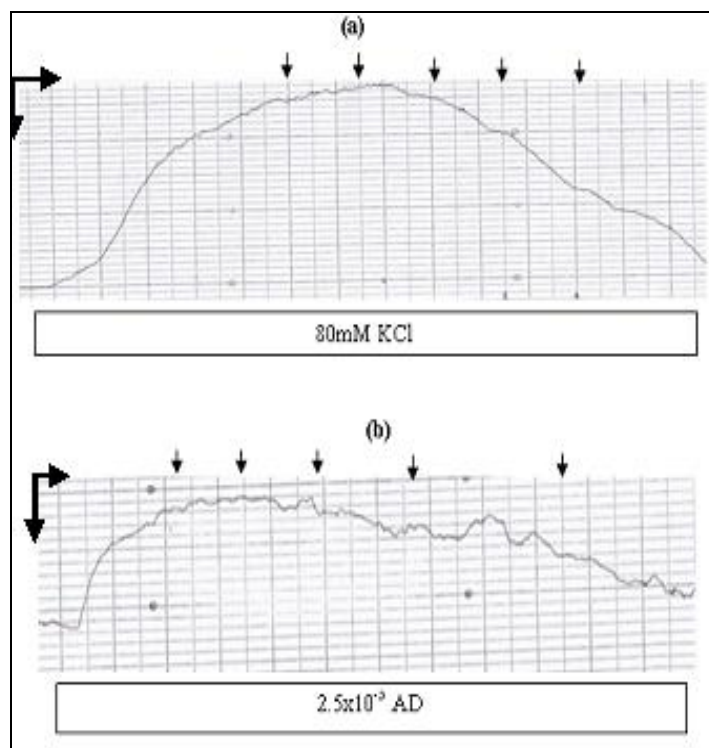
Plants species used	Local Names/ethnicity	Families	Part used	Mode of preparation	Mode of administration
<i>Abrus precatorius</i>	Mudepu (Masango, Baloubou)	Papilionieae	Leaf	Decoction	Drink
<i>Allium sativum</i>	Ail	Liliaceae	bulb	Decoction	Drink
<i>Annickia chloranta</i> (Oliv.)Setten& Maas	Mfôl (Fang)	Annonaceae	Stem bark	Decoction	Drink
<i>Annona muricata</i>	Corossolier	Annonaceae	Leaf	Maceration	Ingurgitation
<i>Anthocleista vogelii</i> Planch.	Teng mavassa (Massango),	Loganiaceae	Stem bark	Decoction	Drink
syn <i>Anthocleista macrantha</i> Gilg	Ayinebe (Fang)				
<i>Capsium frutescens</i> A.Br. & Bouché	Nungu (Masango)	Solanacea	seed	Maceration	ingurgitation
<i>Cleistopholis patens</i> (Benth.) Engl.&Diels.	Doundzou (Masango), Ovôc (Fang)	Annonaceae	Stem bark	Decoction	Drink
<i>Cola acuminata</i> (P.Beauv.)Schott& Endl.	Colatier	Sterculiaceae	fruit	Maceration	Ingurgitation
<i>Copaifera religiosa</i>	Murei (Punu)	Cesalpiniaceae	Stem bark	Decoction	Drink
<i>Cymbopogon citratus</i> . Stapf.	Citronelle	Poaceae	Leaf	Decoction	Drink
<i>Guibourtia tessmannii</i> (Harms) J.Léonard	Kevazingo	Cesalpiniaceae	Stem bark	Decoction	Drink
<i>Lygopodium microphyllum</i> (Cav.)R.Br.	Magoï (Punu)	Schizaeaceae	Leaf	Decoction	Drink
<i>Momorcica charantia</i> . L.		Cucurbitaceae	Leaf	Decoction	Drink
<i>Musanga cecropioides</i> R.Br.ex Tedlie.	Divala (Punu), Parassolier	Moraceae	Stem bark	Decoction	Drink
<i>Persea gratissima</i>	Avocatier	Lauraceae	Leaf	Decoction	Drink
<i>Picalima nitida</i> . (Stapf) T. Durand & T. Durand	Ebam (Fang), Dugundu (Masango)	Apocynaceae	Leaf Fruit	Decoction	Drink
<i>Piper guineense</i> . Schumach. & Thonn.	Abô-Me-Nzang-Ndic (Fang)	Piperaceae	Linea	Decoction	Drink
<i>Piper umbellatum</i> .L.	Abô-Me-Nzang (Fang)	Piperaceae	Linea	Decoction	Drink
<i>Psidium guajava</i> .L.	Goyavier	Myrtaceae	Leaf	Decoction	Drink
<i>Senecio gaboneensis</i>	Budjambu (Masango)	Composeae	Leaf	Maceration	ingurgitation
<i>Sterculia tragacantha</i> Lindl.	Ivostou (Masango), Mundundu (Punu)	Sterculiaceae	Stem bark	Decoction	Drink
<i>Strombosiopsis tetranda</i> Engl.	Mugamba-malungu (Punu)	Olacaeae	Stem bark	Decoction	drink

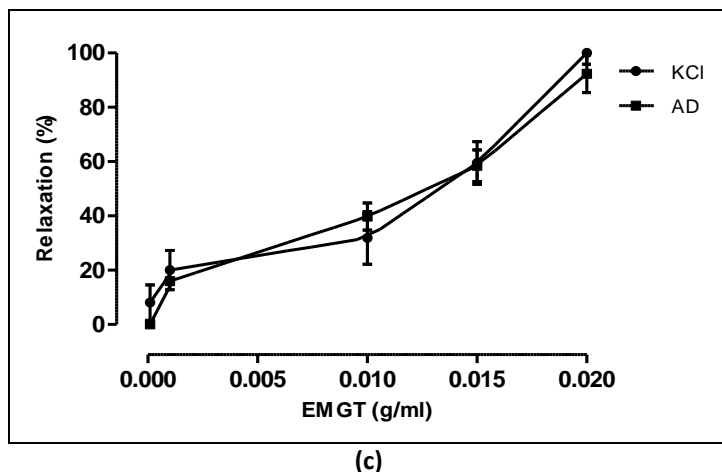
TABLE 2: PHYTOCHEMICAL SCREENING OF STEM BARKS OF *GUIBOURTIA TESSMANNII*

Tests	Results
Fats	-
Sterols	+
Triterpenes	+
Carotenoids	-
Alkaloids	+
Flavonoids	-
Tannins and polyphenols	+
Saponosids	-
Reductor sugars	+

(+) present, (-) absents

Regarding the pharmacological study, Figures 1a and b show typical recordings of the effect of increasing concentrations of the methanolic extract of *G. tessmannii* (EMGT) in guinea-pig isolated intact aorta pre-contracted with the KCl (80 mm) (Fig. 1a) or the adrenalin ( $2.5 \times 10^{-2}$  mg/ml) (Fig. 1b) respectively. Figure 1c represents the mean values of the relaxation induced by EMGT ( $10^{-4}$  g/ml -  $2 \cdot 10^{-2}$  g/ml).

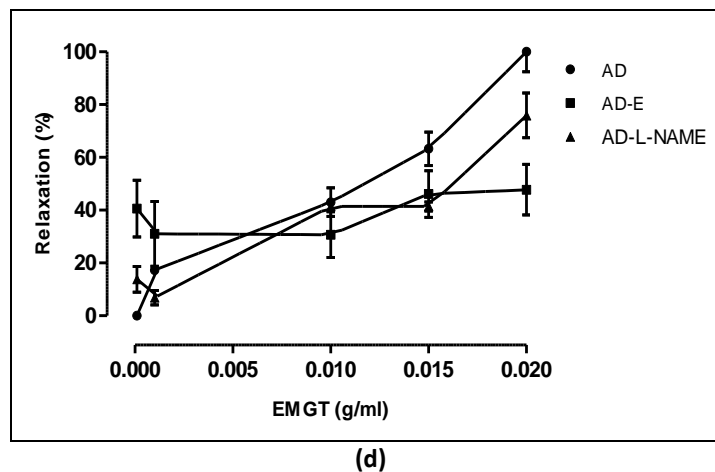




**FIG. 1: VASORELAXANT EFFECTS OF THE METHANOLIC EXTRACT OF *GUIBOURTIA TESSMANII* IN THE GUINEA-PIG ISOLATED AORTA**

Corrected: (a) and (b): Original tracing of EMGT-induced Vasorelaxation of contraction evoked by KCl or adrenalin. EMGT was applied at increasing concentrations ( $10^{-4}$  g/ml -  $2.10^{-2}$  g/ml). Horizontal scale: 30 s; vertical scale: 120 mg. (c) Dose-dependent relaxation induced by EMGT on contractions evoked by KCl (80mM) or Adrenalin ( $2, 5 \times 10^{-2}$  mg/ml).

KCl (80 mM) and adrenalin ( $2, 5 \times 10^{-2}$  mg/ml) elicited a muscular tension of  $5.37 \pm 1.56$  mN and  $3.6 \pm 0.48$  mN respectively in rat isolated aorta. EMGT ( $10^{-3}$  g/ml -  $2.10^{-2}$  g/ml) produced a dose-dependent relaxation of KCl-induced contraction from a minimal relaxation of  $7.03 \pm 4.35$  % ( $p > 0.05$ ) to a maximal one of  $87.14 \pm 3.56$  % ( $p < 0.001$  vs. KCl contraction). Similarly, Ad-evoked contraction was significantly and dose-dependently decreased by EMGT ( $10^{-3}$  g/ml -  $2.10^{-2}$  g/ml) from a minimal relaxation of  $13.91 \pm 0.51$  % ( $p < 0.05$ ) to a maximal one of  $80.05 \pm 6.05$  % ( $p < 0.001$  vs Ad contraction).

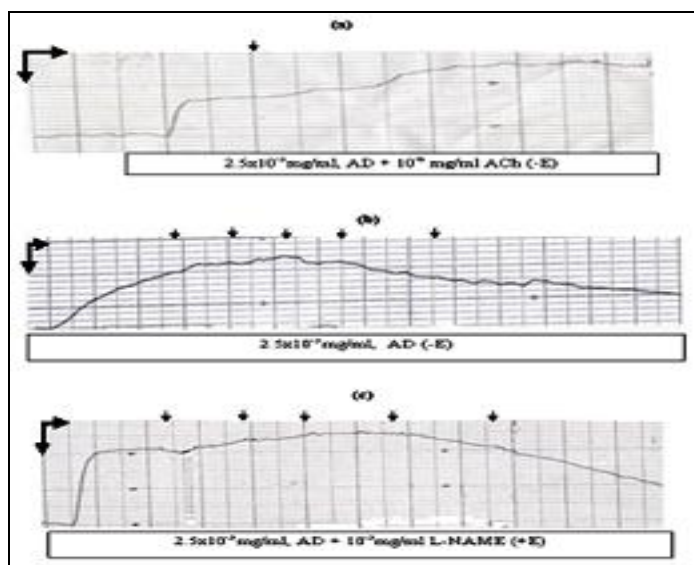


**FIG. 2: VASORELAXANTS EFFECTS OF THE METHANOLIC EXTRACT OF *GUIBOURTIA TESSMANII* IN THE GUINEA-PIG ISOLATED AORTA**

(a) Typical tracing of Contractions induced by Adrenalin ( $2.10^{-5}$  mg/ml) followed by the effect of Ach ( $10^{-5}$  mg/ml). (b) : Vasorelaxation induced by increasing concentrations of EMGT ( $10^{-3}$  g/ml -  $2.10^{-2}$  g/ml) on Adrenalin-evoked contraction in endothelium-denuded aorta. (c): Vasorelaxation induced by increasing concentrations of EMGT ( $10^{-3}$  g/ml -  $2.10^{-2}$  g/ml) on Adrenalin-evoked contraction in the presence of L-NAME ( $10^{-5}$  mg/ml). Horizontal scale: 120mg. vertical scale: 30 s. (d) Dose-dependent relaxation induced by EMGT on contractions evoked by KCl (80mM) or Adrenalin ( $2.10^{-5}$  mg/ml).

With endothelium denuded-aortic rings or with endothelium intact aortic rings and in the presence of L-NAME (**figure 3**), EMGT-induced relaxation of contractions evoked by adrenalin was significantly reduced but not totally abolished. Indeed, the maximal relaxation elicited in denuded aortic strips was  $51.11 \pm 6.83$  vs.  $80.0 \pm 56.05$  (in endothelium intact rings) ( $p < 0.05$ ). In the same way, the maximal relaxation induced by EMGT in the presence of L-NAME was  $38.43 \pm 7.72$  vs.  $80.0 \pm 56.05$  (in the absence of L-NAME) ( $p < 0.01$ ).

**DISCUSSION:** Nowadays, hypertension represents the first cause of mortality among all cardiovascular diseases<sup>8</sup>. Our ethnobotanical investigation conducted at Libreville herbal market showed that in this urban area, in spite of attending hospital, people are still dependent on phytomedicines for the management of hypertension. Indeed, our results revealed that, for one of all Libreville herbal markets and a sample of 29 herbalists, 22 plants species are used for the management of hypertension. This result, following the example of those of JOUAD<sup>3</sup> on ethnobotanical surveys of medicinal plants used to treat diabetes, renal and cardiac diseases in Fez region (Morocco) or



those of N'GUESSAN-KOFFI<sup>4</sup> on plants used for the management of arterial hypertension conducted at Abgville (Ivory-Coast), confirmed the strong position of the phytotherapy and traditional medicine in African societies and then, the needs of scientific studies.

Regarding the parts of the plants used, the modes of remedies preparation and administration, our results revealed that leaves are the most used. This is important for the conservation of these species since the collection of leaves from plants causes little damage to plant populations<sup>9</sup>. Almost the remedies recorded were mono-specific. Since, many fatal accidents due to phytomedicine are a result of improper mixtures<sup>10</sup>. It appeared clearly, in accordance with NGUESSAN KOFFI<sup>4</sup> that the use of

mono-specific receipts would be less dangerous and more appropriate for the health of large populations. Among the 22 identified plant species, 50% of them are well documented for the management of hypertension: *Abrus precatorius*, *Allium sativum*<sup>3, 4</sup>, *Piper umbellatum*, *Momordica charantha*, *Annona muricata*, *Musanga ceropioides*, *Cola acuminata*, *Cymbopogon citrates* *Persea Americana*<sup>4</sup>, *Picralima nitida*<sup>11, 2, 12</sup>, and *Guibourtia tessmannii*<sup>13, 14</sup>. Among all species recorded and well documented for their traditional use in the management of hypertension, *G. tessmannii* was the most used species. However, there are few reported experimental studies on this plant. The plants recorded are known to have beneficial properties arguing for the treatment of several ailments (**Table 2**).

**TABLE 2: PHYTOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF PLANTS**

Plants species	Phytochemistry	Pharmacology	Literature
<i>Abrus precatorius</i>	Glycoside. Triterpenoid saponin. Flavonol glycoside;	Antitubercular and antiplasmodial. Antidiabetic effect. Immunopotentiating activity. Anti-inflammatory activity. Antimicrobial activity.	26, 27, 28, 29, 30, 31, 32,33
<i>Allium sativum</i>	Iode. Phenylpropanoids.	Lowers cholesterol. Fumigant activity. Vasorelaxant effect	34, 35, 36, 3,4
<i>Annona muricata</i>	Alkaloids. (isoquinoline). Volatils compounds. Essential oil	Antiparasitic activity. Cytotoxicity and antileishmanial activity.	37, 38, 39, 40, 41,42
<i>Cola acuminata</i>	Phenolic compounds	Hypotensive activity.	43, 4
<i>Cymbopogon citratus</i>	Alcohols (linatol,geraniol). Essentiel oil.	Diuretic effect. Antimicrobial activity. Hypoglycemic and hypolipidemic effects. Neurobehavioral effect.	25, 44, 45, 46,47
<i>Guibourtia tessmannii</i>	Stilbene glycosides. Asebotin. Chalconoid, stilbenoid glycosides.	Hypotensive activity.	13, 48, 14,15
<i>Momordica charantia</i>	Polysaccharides. Saponin. Phenolic compounds. Curcubinate Triterpenoids.	Hypoglycemic activity; Antioxidant activities. Antihyperglycemic effects. Antimicrobial activity.	49,50,51,52,53,54
<i>Musanga ceropioides</i>	Phenolic compounds. Procyanidin. Pentacyclic triterpene. Saponin	Vasodilating properties, Hypoglycemic and antidiabetic activities. Hypotensive mechanisms.	55, 56, 57, 58, 59, 60,61
<i>Persea Americana</i>	Flavonoids	Anti-icteric and hepatoprotective activity. Hypotensive activity.	62, 4
<i>Picralima nitida</i>	Alkaloids, (akuamin, picranitine, picraline)	Anti-inflammatory and analgesic actions. Antimicrobial property. Depression effect.	63, 64, 65, 66, 67, 68
<i>Piper umbellatum</i>	Essential oils. PPolyphenol. Glycoside.	Antiplasmodial activity. Antioxydant activity. Analgesic. Inhibitor effect on myotoxic phospholipases A <sub>2</sub> from snake venoms.	69, 70, 71, 72, 73

Phytochemical studies of the plant extract revealed the presence of many bioactive compounds such as tannins, flavonols and sugars which have been previously described<sup>14, 15</sup>. The results of the preliminary pharmacological investigations showed that the methanolic extract of *Guibourtia tessmannii* (EMGT) exerted vasorelaxant effect in the isolated aorta pre-contracted with KCl or with adrenalin.

Contraction of vascular smooth muscle cells induced by adrenalin is characterized by phasic and tonic responses due to the activation of  $\alpha$ -adrenoceptors leading respectively to the mobilization of intracellular calcium from internal stores and an intensification flux from extracellular medium<sup>16, 17, 18</sup>. Whilst, smooth muscle contraction evoked by KCl is essentially due to the intensification of calcium influx through voltage-dependent Ca<sup>2+</sup> channels<sup>16, 19</sup>.



The fact that EMGT inhibited adrenalin- or KCl-evoked contraction with almost similar potency suggests that, the observed effect may be due to a mechanism involving an inhibition of calcium influx and/or an inhibition of intracellular calcium release. This hypothesis gives rise to some questions related to the mechanism underlying such an action.

Furthermore, it is well known that much pathology such as hypertension may be the result of structural and functional alterations of vascular endothelium which are important in the mechanisms that determine arterial blood pressure<sup>20</sup>.

In a study to investigate the possible involvement of NO in plant extract-induced relaxation, removing endothelial layer mechanically or with a pretreatment by L-NAME, a competitive inhibitor of NO-synthetase<sup>18, 21</sup>, significantly reduced vasorelaxant activity induced by EMGT in adrenalin-evoked contraction. However, EMGT-evoked vasorelaxation was not completely abolished.

This suggests the involvement of an endothelium-dependent mechanism NO signaling pathway and an endothelium-independent mechanism. Tannins, alkaloids, polyphenols and saponosides are well known for their bioactive properties which may be related to cardiovascular affections. Indeed, numerous studies have been reported for tannins: antioxidant and spasmolytic properties on smooth muscle<sup>22</sup>, for polyphenols: diuretic action<sup>4</sup>, antioxidant vasodilator activities<sup>23, 25</sup> and for saponosides: diuretic action<sup>25</sup>. Vasorelaxant action of EMGT could result in the action of some of these chemical compounds. This study could scientifically support the use of *G. tessmannii* for the treatment of arterial hypertension in folk medicine.

Overall, folk medicine may provide many efficient remedies that are able to manage efficiently arterial hypertension. Thus, further laboratory experimental studies are required in order to highlight mechanisms of action and to prevent side effects of medicinal plants use. Studies of other receipts using *G. tessmannii* used by populations in other Central African countries may yield insights into other antihypertensive remedies and may represent a potential source of antihypertensive agents.

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