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Phytochemical screening and pharmacological effect of aqueous leaf extracts of *Tridax procumbens* L. (Asteraceae) used in traditional treatment of high blood pressure in Côte d'Ivoire

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Abstract
High blood pressure (HTA), which was once considered a rare disease in Black Africa, is today experiencing a dazzling and worrying progression mainly due to its complications. In Africa, HTA affects about 10 to 17 % of the population. In Côte d'Ivoire, this prevalence is estimated to be between 7 and 13 %. Because of the generally high costs of antihypertensive treatments based on modern medicines, in the context of the valorization of traditional medicine, we evaluated the pharmacological effect of the leaf decoctate of *Tridax procumbens*, a plant used for the treatment of high blood pressure. Phytochemical trials carried out to the leaves of this plant revealed the presence of stress and polyterpenes, polyphenols, flavonoids, tannins, saponosides and alkaloids. The pharmacological study showed that leaves aqueous extract of *T. procumbens* is endowed with hypotensive properties which are linked to the phytochemical constituents found in the leaves.

Keywords: *tridax procumbens*, hypertension, leaf extract, pharmacology, phytochemistry

1. Introduction

The increasing prevalence of hypertension and their complicity, modern therapeutics offers a wide range of antihypertensive drugs. Unfortunately, the costs of these remedies are often beyond the reach of third world populations [1, 2]. In Africa and particularly in Côte d'Ivoire, hypertension is difficult to cure and today it is of great concern to the health authorities. In addition to cultural reasons, most hypertensive patients turn to traditional medicine for economic reasons [3, 4]. In this area, there are many plants known to be antihypertensive such as bridal foregoing, *Ziziphus mauritiana*, *Persea americana*, *Western anacardium*, *Annona muricata*, *Mangifera indica*, *Lantana camara* and *Tridax procumbens* [5-7]. It is now widespread in all regions of the world and particularly in Côte d'Ivoire. *T. procumbens* development as a radial species along the paths and as a weed of annual and perennial crops [8-10]. This plant usually has a prostrate and erect habit. The root consists of a steering pivot. The stem is cylindrical solid. The leaves are opposite, simple. The flowers are assembled in capitals. These capitals are solitary. The fruits are conical achenes 3 mm high. *T. procumbens* is used in traditional African and Asian pharmacopoeias like purgative, laxative, analgesic, anemia, arthritis, etc. It is used in the treatment of diseases such as diabetes, high blood pressure. Leaf extracts are anti-inflammatory, antioxidant, antipyretic, Hepatoprotective, antimicrobial, hypocholesterolemic and weight reduction [6, 11, 12]. Among the biological activities, flavonoids have actions against free radicals.

The purpose of this study is to determine the main chemical constituents of *T. procumbens* leaves and to evaluate the pharmacological effects of the aqueous extract (decoction) on rabbit blood pressure.

2. Materials and methods

2.1 Plant material

The plant material consists of the leaves of *T. procumbens*. This plant was harvested near Félix Houphouet Boigny University (Côte d'Ivoire) and identified by the technician Yapo Assi Jean of the National Center of Floristics where is preserved a sample of this species Harvested for the first time. (Herbarium No. 147855 of July 18, 1979). The leaves of the plant were washed with tap water abundantly and then dried in the open air before being reduced to powder with the aid of a micro-grinder.

2.2 Animal material

Rabbits belonging to the species *Oryctologus cumins* (Leporidae) were used to study the effects of aqueous extracts on blood pressure. Their weight varies between 2 and 3.1 Kg. These Rabbits come from farms in the vicinity of Abidjan (Côte d'Ivoire). They were acclimatized for two weeks to the animal house of Biosciences Training Unit of Félix Houphouet Boigny University.

2.3 Phytochemical study

The phytochemical study was performed to identify the major groups of chemical constituents responsible for

pharmacological activities. Three methods of extraction were used, namely extraction by successive solvents (chloroform and methanol), infusion and the method recommended in traditional medicine (decoction).

Sterols et polyterpenes analysis were performed with reaction of Libermann [13]. The reaction with ferric chloride was used for the investigation of polyphenols and flavonoids group are dtected by the reaction with cyanidine [14]. For the tannins the highlighting was made thanks to the reaction of Stiasny. The reagent was prepared by mixing 10 ml of formalin 30 % with 5 ml of pure hydrochloric acid. The free or combined known compounds are demonstrated by the Borntraeger reaction [15]. The investigation of saponosides is based on their property to be foamed after agitation in aqueous solutions [16]. Alkaloids were highlighted using methods using the Dragendorff and Bouchardat reagents [17].

2.4 Pharmacological study

The crude aqueous extract obtained after lyophilization of the leaves decoctate of *Tridax procumbens* was used for the pharmacological study. It used at 15, 38, 69 and 120 mg/Kg of body weight. Adrenaline (Prolabo, France), a neuromediator belonging to the orthosympathetic system was used as a hypertensive substance at 0.2 µg/Kg of body weight. Diltiazem (Synthelabo, France), a calcium antagonist belonging to the class of benzothiazepines was used as antihypertensive. A solution of Mac Ewen consisting of NaCl (130), KCl (4.8), $CaCl_2$ (2.21), Na_2HPO_4 (1.18), $NaHCO_3$ (11.9), $MgCl_2$ (0.24), anhydrous glucose (2.5) This solution was used at a pH of 7.4 and was also used to dilute the reference products which are adrenaline (Adr) and diltiazem (Dtz). Three doses were used: 136, 360 and 675 µg/Kg of body weight. The arterial pressure of the rabbit carotid artery was recorded by a Ludwig manometer composed of a U-shaped tube with both branches containing mercury [18]. An overpressure is achieved before intubation. The animal was anesthetized by intra-peritoneal injection of ethyl methane 40% at 1.0 g/Kg of the body. After a transverse incision of the vein, a catheter adapted to a syringe containing a heparinized Mac Ewen solution was introduced into the vein. The second ligature was tightened on the catheter. The tourniquet was then removed and some heparinized physiological solution was injected in order to prevent coagulation of the blood in the catheter. The carotid was ligated downstream to prevent blood from flowing back from the head of the animal. The catheter connected to the intubation tube filled with heparinized physiological solution is introduced into the artery in the direction of the heart. Finally, the cylinder motor was switched on and the reference level of the pressure in the pressure gauge was written on the recording paper. The drop of the writing pen corresponds to the reference blood pressure of the rabbit and the normal pattern of changes in arterial pressure of the carotid artery was thus recorded.

3. Results and discussion

3.1 Phytochemical study

Phytochemical analysis of *Tridax procumbens* leaf extracts revealed the presence of alkaloids, flavonoids, tannins, sterols, polyterpenes in the extracts (Table 1). On the other hand, Spaniards' and known compounds are absent in chloroform and methanol extracts. This qualitative phytochemical study shows that all chemical groups identified at the leaf level of *Tridax procumbens* are extractable by water, as seen with the infused and decoctate. It also shows that the temperature has no significant effect on these compounds, since they are found both in the infused and in the decoctate. The presence of saponosides at leaf level could be related to sterols and polyterpenes. In fact, saponosides are heterosides whose nucleus is either sterol C_{27} (spirostane) or pentacyclic triterpene [3, 19]. It should be noted, however, that other stars and polyterpenes may well be present in the plant without being bound to the saponosides. The leaves had a higher catechin, gallocatechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocathe. Flavonoids present in the leaves of *Tridax procumbens* have actions against free radicals. These flavonoids fight inflammations, allergies, platelet aggregations, microbes, ulcers, viruses, tumors and hepatotoxins [6, 7, 20]. Kaempferol-3-O-rutinoside-type flavonoids with hypotensive activities were extracted from the leaves of *Tridax procumbens* by Amhad *et al.* [21]. The same applies to apigenin-type flavonoids which possess antibacterial, anti-inflammatory, diuretic and hypotensive properties [22, 23]. It has been shown that catechins are antimicrobial, antiallergic, anticancer, antidiabetic and antihypertensive, antioxidant [24]. It has been extracted from the leaves of *Tridax procumbens* of alkaloids of decline types which is an antiarteriosclerotic, an antibacterial and an antihypertensive. Quercetin has been reported to have antidiabetic, anti-cancer, antihypertensive and anti-arthritic properties [25, 26]. The presence of tannins appears to be related to the recurrent use of *Tridax procumbens* in wound dressing and the treatment of diarrhea due to their astringent power [6]. In addition, phytochemical analysis shows that the decoction and infusion of *T. procumbens* leaves, traditionally used forms, are richer in substances than chloroform and methanol extracts.

Table 1: Phytochemical screening of leaves extract of *Tridax procumbens*

Phytochemical compounds	Chloroform	Methanol	Infused	Decoction
Sterols and polyterpenes	+	+	+	+
Polyphenols	+	+	+	+
Flavonoids	+	+	+	+
Quinone substances	-	-	+	+
Saponosides	-	-	+	+
Catechin tannins	+	+	+	+
Gallic tannins	-	+	+	+
Alkaloids	+	+	+	+

(+) presence; (-) absence

3.2 Pharmacological study

Figure 1 shows the effects of *Tridax procumbens*, at increasing doses, on arterial hypertension induced by an adrenaline concentration (Adr) equal to 0.2 µg/Kg of body weight. Adr at 0.2 µg/Kg of body weight induces, after a latency period, a hypertension which lasts about 1 min 30 s. This increase in blood pressure reaches a value of 200 mm Hg (Figure 1.1). In the presence of *T. procumbens* (Trip), at increasing doses ranging from 15 to 120 mg/Kg body weight, Adr at 0.2 µg/Kg induces less and less severe duration and intensity of hypertension, which are often followed by sustained hypotension as shown in Figure 1 (2-5). Hypertension fades rapidly when the Trip dose increases (Figure 2). Thus, rabbits which consume Trip at 120 mg/Kg body weight develop a blood pressure of 20 mm Hg while those which consume 15 mg/Kg of Trip body weight have 70 mm Hg of blood pressure. These results suggest that blood pressure is inversely proportional to the Trip dose consumed.

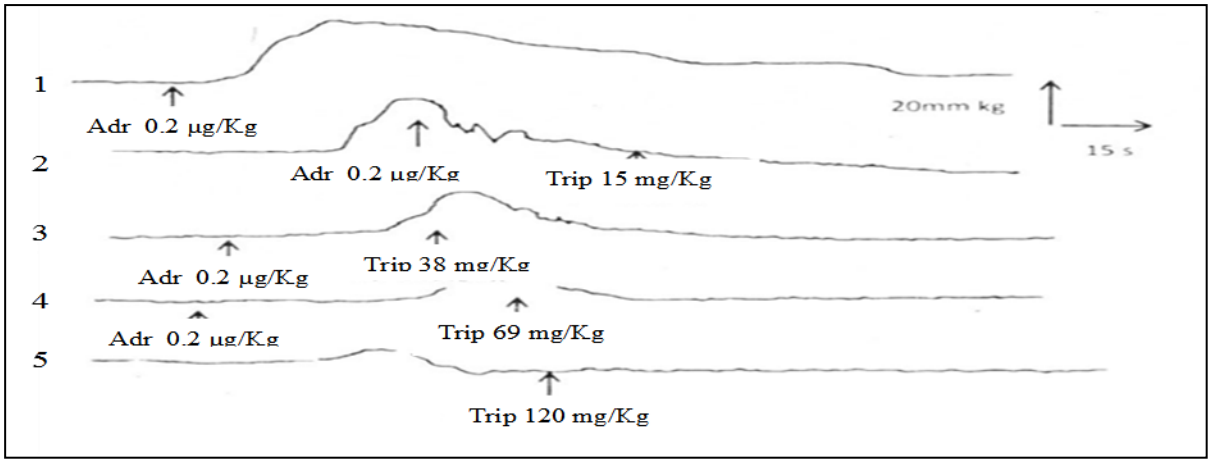

Fig 1: Effect of *Tridax procumbens* aqueous extracts on adrenaline-induced hypertension in rabbits

1, effect of adrenaline (Adr) 0.2 µg/Kg body weight; 2 to 5, adrenaline effect (Adr) 0.2 µg/kg body weight followed by the effect of extract of *Tridax procumbens* (Trip) at 15, 38, 69 and 120 mg/Kg body weight.

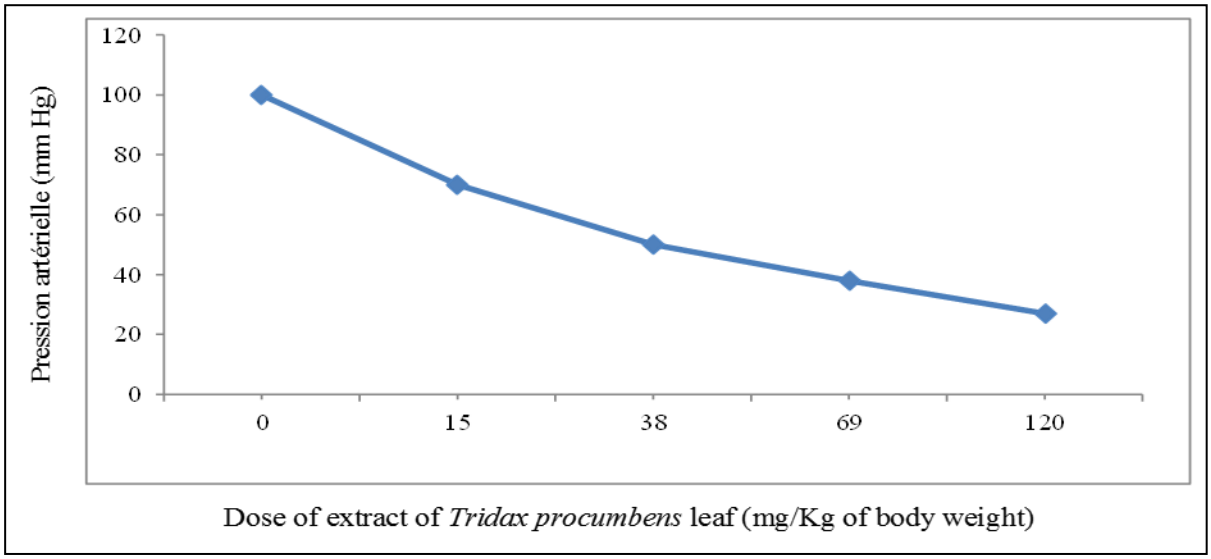

Fig 2: Decrease in adrenaline-induced hypertension in rabbits as a function of dos of *Tridax procumbens*

Trip seems to have a hypotensive effect. A comparison of its effect with a hypotensive agent, diltiazem (Dtz) belongs to the class of drugs called calcium antagonists. It is used alone to treat high blood pressure [27]. Dtz is an antihypertensive agent belonging to nifedipine and verapamil, the anti-calcium compounds. It works by releasing blood vessels and reducing the workload of the heart.

Figure 3 illustrates the effects of diltiazem (Dtz) at increasing doses of Adr hypertension. This hypertension reaches its maximum value of 150 mmHg (Figure 3.1). In the presence of Dtz, at increasing doses ranging from 136 to 675 µg/Kg body weight, Adr induces transient hypertensions of decreasing values, followed by sustained hypotension under the effect of Dtz, as shown in Figure 3 (2-5). Figure 4 confirms that high doses of Dtz lowers the blood pressure caused by Adr as in the case of Trip. This curve shows that the decrease in induced hypertension is less important for concentrations of Dtz of less than 360 µg/Kg of body weight than for higher concentrations.

This dose seems to be the minimal to use to bring down the blood pressure in the rabbit. The crude leaf extract of *T. procumbens* inhibits adrenaline-induced arterial hypertension. This dose-dependent inhibition is comparable to that induced by Dtz on the same hypertension. Since Dtz is a known antihypotensive agent, it is deduced that *T. procumbens* is a potential antihypertensive agent. Therefore, the use of this plant in the treatment of high blood pressure by health practitioners is fully justified. This hypotension confirms the antihypertensive properties of *T. procumbens* [7, 28]. Indeed, unlike diltiazem which causes sustained hypotension at all doses, the raw extract of *T. procumbens* induces labile hypotension except at high doses. By extrapolation, the effects of *T. procumbens* would probably reflect the presence of cholinomimetic substances in the leaf decoctus of this plant, as was shown with the crude leaf extract of *Mareya micrantha* [29], *Heliotropium* (*Gossypium barbadense*) [23]. The species is characterized by the presence of an insecticidal organism. Therefore, their wide use in traditional African medicine against high blood pressure seems to be indispensable [11]. Indeed, they are accessible to all the population because cheaper than modern medicines. Moreover, the pharmacological property of the plants extract would be linked to the conjugated actions of the phytochemicals that it contains such as the alkaloids, flavonoids, sterols, saponosides and tannins. Saponosides and flavonoids by their diuretic effect effectively combat high blood pressure [30].

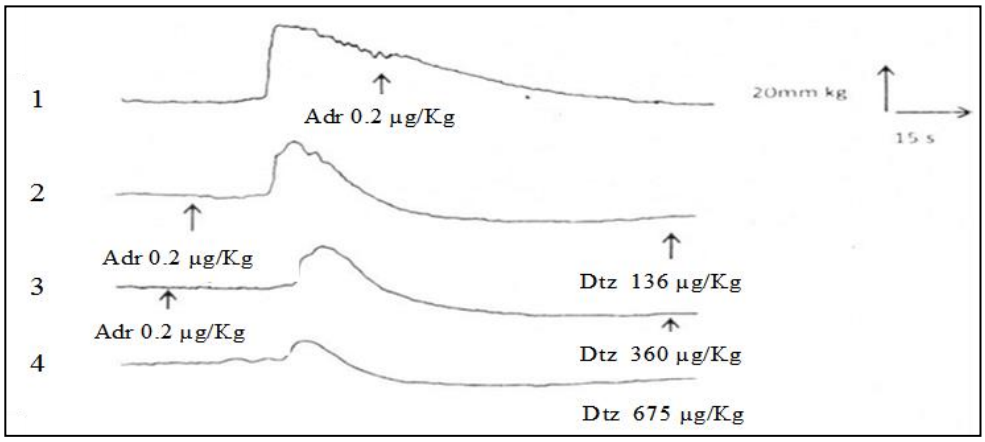

Fig 3: Effect of diltiazem on hypertension adrenaline-induced in rabbits

1, effect of adrenaline (Adr) 0.2 µg/Kg body weight; 1 to 4, adrenaline effect (Adr) 0.2 µg/kg body weight followed by the effect of extract of diltiazem (Dtz) at 136, 360 and 675 µg/Kg body weight

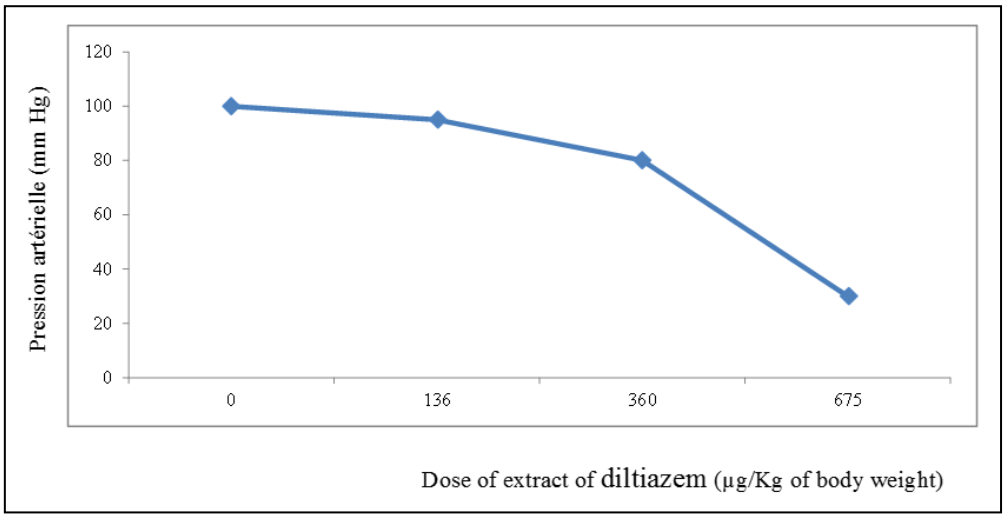

Fig 4: Decrease in adrenaline-induced hypertension in rabbits as a function of dose of

This study demonstrated that high blood pressure can explore the hypotension potential of *T. procumbens*. Thus, people can be encouraged to include the decoctate of this plant in their diets because of its protective against various diseases such as hypertension.

4 Conclusion

This study contributes to the valorization of medicinal plants against high blood pressure which has become a public health problem in Africa. Phytochemical analysis shows that the decoction or infusion of the leaves of *T. procumbens* is the

best form to be administered because it allows maximum extraction of the active substances. The pharmacological study shows that the crude extract of *T. procumbens* inhibits adrenaline-induced arterial hypertension. The hypotensive properties of this plant are related to chemical substances such as flavonoids, alkaloids, tannins, sterols and especially the saponosides and flavonoids it contains. Furthermore, researches can still be carried out to develop of *T. procumbens*-based drugs in the control of hypertension.

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