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Original Article

Antihypertensive Activity of Different Fractions of *Tridax Procumbens* Crude Aqueous Extract in Wistar Rats

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Abstract

Tridax procumbens is a medicinal plant used in traditional medicine for the treatment of hypertension but only few pharmacological data are available on its antihypertensive effect. The objective of this work was to assess the effect of *Tridax procumbens* extracts on arterial pressure of rats made hypertensive. Crude aqueous extract of aerial part of *Tridax procumbens* was fractioned by liquid-liquid chromatography method successively with cyclohexane, dichloromethane and ethyl acetate. A chemical screening was performed using thin layer chromatography. Hypertension was induced in rats by 7-days administration of N (G)-Nitro-L-Arginine-Methyl Ester (L-NAME) at 20mg/kg and fractions were then administrated to rats for the 7 following days at 30 mg/kg. Four main fractions (Dichloromethane, ethyl acetate, micellar and aqueous) were obtained from the crude extract. Altogether, alkaloids, coumarins, flavonoids, tannins, lignans, saponins, anthracenes, terpenes and sesquiterpenes, triterpenes compounds and essential oils were detected in the fractions. L-NAME treatment resulted in an increase in mean arterial pressure (MAP) from 107±7 mmHg to 145±7 mmHg. Ethyl acetate and dichloromethane fractions induced a significant reduction of MAP from 145±7 mmHg to respectively 110±2 and 117±3 mmHg. In view of these data, further studies must be conducted on these two fractions to identify the active molecules and their action mechanism.

Keywords: Hypertension, medicinal plant, L-NAME, *Tridax procumbens*.

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Introduction

Arterial hypertension, the main risk factor of cardiovascular diseases, is an important health problem worldwide. In Africa, the overall prevalence rate of hypertension in 2010 has been estimated to 30.8% (Adeloye and Basquill, 2014).

In Benin, a West African country, this prevalence was 27.9% in 2008 (Houinato *et al.*, 2012). Because of the relatively high cost of the treatment of this pathology, a large proportion of Benin population, suffering from hypertension, refers to medicinal plants for their treatment.

Tridax procumbens, is a medicinal plant used in Benin and in several other countries for the treatment of many diseases. This plant is used in traditional medicine in the treatment of diarrhoea, dysentery, typhoid fever, cough, asthma, epilepsy (Ali *et al.*, 2001; Mann *et al.*, 2003). Moreover, wound healing, hepato-protective, anti-inflammatory, antidiabetic, anti-parasitic, antibacterial, antioxidant, haemostatic and immunomodulatory effects of *Tridax procumbens* have also been reported (Vilwanathan *et al.*, 2005; Awasthi *et al.*, 2009; Vyas *et al.*, 2004, Abhijit *et al.*, 2014; Bhagwat *et al.*, 2008; Zhelmy *et al.*, 2009; Kale *et al.*, 2008; Habila *et al.*, 2010; Malik *et al.*, 2012; Regina and Mohamed, 2014; Dhanabalan *et al.*, 2008).

In addition to these pharmacological properties, *Tridax procumbens* is one of the medicinal plant used in the treatment of hypertension. However, only a few studies have been dedicated to the antihypertensive activity of *Tridax procumbens*. In normotensive rats, it has been shown that administration of *Tridax procumbens* extract induced a reduction of blood pressure associated to heart rate decrease (Salahdeen *et al.*, 2004). In vitro, a vasorelaxation effect of *Tridax procumbens* has been reported (Salahdeen and Murtala, 2012) but investigation of the effect of this plant on hypertension in human or in an animal model has never been reported. In a previous study, we have observed that crude aqueous extract of aerial part of *Tridax procumbens* induced a normalization of blood pressure in rats made hypertensive by L-NAME administration (unpublished data). In view of these data, we hypothesised that fractioning the crude extract and investigation of the pharmacological activity of resulting fractions, could lead to the selection of active fractions and latter to the identification of active molecules and their mechanism of action. Thus, in the present work, the antihypertensive activity of different fractions obtained from the crude aqueous extract was investigated.

Materials and Methods

Plant Material

Tridax procumbens aerial part was collected in the town of Cotonou, Republic of Benin. Plant was identified and authenticated at the Herbier National under the number AA6394/HNB. Plant material was washed, shade dried (temperature 20-25°C for two weeks) and pulverized using a MIKACHI-MK 1861 AP blender. The powder was stored at room temperature until used.

Extraction Procedure

425 g of the powder was added to 1000 mL of distilled water and the mixture was boiled for 30 minutes. After cooling, the decoction was filtered using Whatman paper N° 1 (Whatman international Ltd; Maidstone, England) and then concentrated by evaporation at 60°C under reduced pressure using a rotary evaporator Rotavapor Buchi R-3 (Sigma-Aldrich, Germany) to obtain the crude aqueous extract.

The crude aqueous extract was then submitted to a liquid-liquid extraction using successively cyclohexane, dichloromethane and ethyl acetate. 50 g of the aqueous extract were dissolved in 500 mL of distilled water and introduced in a 2L-conical flask. 500 mL of cyclohexane was added to this solution and all the content of the flask was well stirred and allowed to stand for phase separation. The organic phase was collected and this process was repeated three times. After cyclohexane, the same procedure was used with dichloromethane and then ethyl acetate. For each of these solvents, the organic phase collected was evaporated at 30°C on the rotary evaporator. The residual aqueous phase was divided into two layers. One more pasty (micellar) and the other more liquid and blackish.

They were also evaporated at 70°C on the rotary evaporator under reduced pressure.

Phytochemical Analysis

The phytochemical analysis of the different fractions was performed using the thin layer chromatography as described by Wagner and Bladt (2001)**.** 5 mg of each extract were dissolved in 1mL of an appropriate solvent (mixture of methanol/water (1: 1), dichloromethane and ethyl acetate). Chromatographic Silica gel 60 F254 plates (Merck) were then loaded with 10 µL of each fraction and the migration was performed using an appropriate solvent system according to each chemical group. Thus, for coumarins, flavonoids, tannins, triterpenes and anthocyanic compounds, ethyl acetate/formic acid/acetic acid/water (100: 11: 11: 26) has been used. The migration solvent used for alkaloids, anthracenes, and glycosides was ethyl acetate/methanol/water (100: 13.5: 10). For respectively, lignans, terpenes and sesquiterpenes, saponin, naphthoquinones, and essential oils, migration solvents used were respectively chloroform/methanol/water (70: 30: 4), chloroform/methanol/water (65: 25: 4), chloroform/acetic acid/methanol/water (64: 32: 12: 8), toluene/formic acid (99:1), and toluene/ethyl acetate (93: 7).

Anti-Oxidant Activity

A qualitative free radical scavenging activity was checked out by chromatographic method. Each fraction was reconstituted in methanol (10 mg/mL). 10µL of this solution were spotted on the plate and after migration using ethyl acetate/methanol/water (80: 12: 10) as solvent, the plate was sprayed with 1, 1-diphenyl-2-pycrylhydrazyle (DPPH). The presence of antioxidant substances was revealed by apparition of a yellowish color in a purple bottom.

Animal Experiments

Experimental Groups

12-15 weeks old male wistar rats weighing 200 g – 250 g, were used. They were maintained in standard environmental conditions (22 to 25°C, 12h dark/light cycle) and had free access to food and water. All the experimental procedures using these animals have been performed in accordance with institutional ethical recommendations.

Rats were assigned to seven (7) groups of five animals as follows:

Control group of rats which received distilled water from day 1 to day 14.

L-NAME Group

Rats were treated with L-NAME (20mg/kg) from Day 1 to Day 7 and received distilled water from day 8 to day 14.

Four (4) Groups (L-NAME-Fraction)

In these groups, following 7-day-administration of L-NAME, rats of each group were treated with one of the fractions of *Tridax procumbens* (30 mg/kg of body weight) for 7 days.

L-NAME-Captopril Group

Animals in this group were treated with Captopril at 100mg/kg of body weight from day 8 to day 14 after L-NAME administration from day 1 to day 7.

All substances (L-NAME, captopril and fractions) have been administrated orally (gavage) to rats.

Blood Pressure Measurement

Rats were anesthetized by intra peritoneal injection of thiopental (40mg/kg of body weight). Left carotid artery was catheterized and blood pressure was measured by invasive method as previously described (Awede *et al.*, 2010).

Statistical Analysis

Blood pressure data were presented as Mean ± SEM (standard error of mean). Data were analyzed using GraphPath Prism 4 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 Extract and Fractions

From 425 g of *Tridax procumbens* powder, 146.10 g of crude aqueous extract have been obtained. The extraction rate was thus 34.38%.

As shown in table 1, liquid-liquid extraction of the crude aqueous extract (50 g) lead to five (5) fractions: cyclohexane (C), dichloromethane (DM), ethyl acetate (EA), aqueous (A) and micellar (M) fractions. The extraction rate of the cyclohexane fraction was so low (0.24%) that this fraction was not taken in account for the rest of the study.

Table 1: *Tridax procumbens* fractions and extraction rate of the liquid-liquid partition.

Fractions	Extraction rate (%)
C	0,24
DM	1,6
EA	2,9
A	58,86
M	25,56

C, DM, EA, A, M represent respectively Cyclohexane, Dichloromethane, Ethyl acetate, Aqueous and the micellar fractions obtained from the crude aqueous extract of *Tridax procumbens*.

Phytochemistry

Table 2 shows the chemical compounds detected in the four main *Tridax procumbens* fractions. Coumarins, saponins and anthracenes were found in all fractions. Alkaloids and flavonoids were detected in both dichloromethane and ethyl acetate fractions. Lignans, tannins, terpenes and triterpenes were detected only in ethyl acetate fraction whereas essential oils were found in dichloromethane fraction.

Table 2: Phytochemical Analysis of the different fractions of *Tridax procumbens.*

Fractions Chemical group	DM	EA	A	M
Alkaloids	++	+	−	−
Coumarins	+	++	+	+
Flavonoids	++	++	−	−
Naphthoquinones	−	−	−	−
Anthocyanics	−	−	−	−
Lignans	−	+	−	−
Saponins	+	++	++	++
Anthracenes	+	++	+	+
Terpenes and sesquiterpenes	−	+	−	−
Tannins	−	++	−	−
Triterpenes	−	+	−	−
Glycosides	−	−	−	−
Essentials oils	+	−	−	−

DM, EA, A, and M represent respectively Dichloromethane, Ethyl Acetate, Aqueous and Micellar fractions. (+) detected, (++) abundantly detected, (+/-) weakly detected, (-) absent.

Anti-Oxidant Effect of the Extracts

A radical scavenging activity was observed with all the fractions. Ethyl acetate fraction showed the highest activity.

Effects of Fractions on Hypertension

Figure 1 shows effects of the fractions on rat blood pressure after hypertension induction. 7-days administration of L-NAME induced an increase of mean arterial pressure from 107±7 mmHg to 145±7 mmHg. One week consecutive administration of ethyl acetate and dichloromethane fractions induced a significant decrease of the mean arterial pressure to respectively 110±2 mmHg and 117±3 mmHg. Effects of these fractions were similar to that captopril, the reference drug, as the mean arterial pressure of rats treated with captopril was 114 ± 3 mmHg. Aqueous and micellar fractions were without significant effect on rat blood pressure.

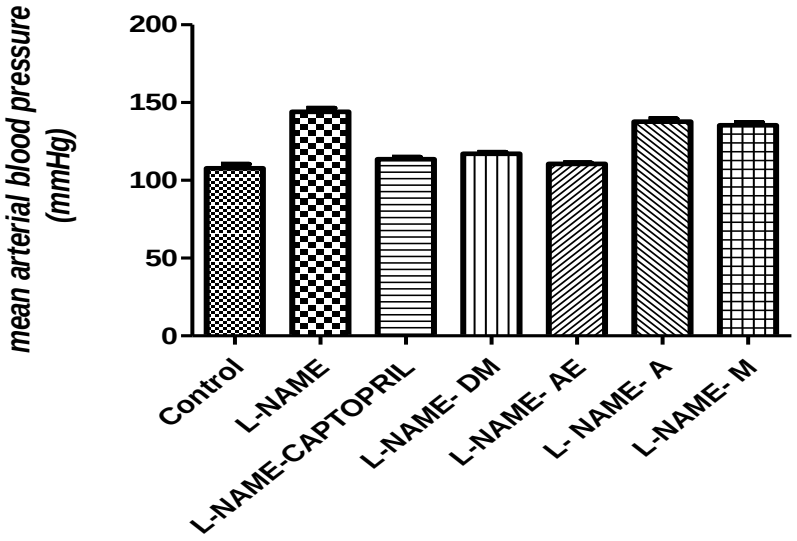

Fig. 1: Effect of *Tridax procumbens* fractions administration on mean arterial pressure (MAP) of L-NAME-hypertensive rats.

L-NAME-Captopril, L-NAME-DM, L-NAME-AE, L-NAME-A and L-NAME-M represent respectively groups of rats treated with respectively captopril, dichloromethane, ethyl acetate, aqueous and micellar fractions after hypertension induction by N (G)-Nitro-L-Arginine-Methyl Ester (L-NAME) administration.

Values of MAP are means ±SEM; n= 5/group.

a: different from control value, $p<0.05$.

b: different from L-NAME group value, $p<0.05$.

Discussion

In the present study, four main fractions were obtained by liquid-liquid partition of *Tridax procumbens* crude aqueous extract. Two, out of the four fractions, induced a normalization of arterial blood pressure in wistar rats made hypertensive by L-NAME administration at 20mg/kg of body weight. The effectiveness of this dose of L-NAME to induce hypertension has been reported previously (Pechanova *et al.,* 1999; Biancardi *et al.,* 2007). 7-days administration of L-NAME at this dose was enough to induce a high blood pressure which persisted at least 7 days more.

Investigation of antihypertensive activity of fractions of *Tridax procumbens* was performed following data showing the antihypertensive effect of *Tridax procumbens* crude aqueous extract (500mg/kg) in the same model of hypertension (unpublished data). Dichloromethane and ethyl acetate fractions were active and had similar effect on blood pressure. The antihypertensive effect of both fractions was observed at a dose almost seventeen fold lower than that of the crude aqueous extract showing that the bioactive molecules were actually concentrated in these fractions.

Phytochemical screening showed that both fractions contained alkaloids and flavonoids, chemical compounds which were not detected in the non-active fractions. These data suggest that alkaloids and, in particular, flavonoids could be involved in the antihypertensive effect of *Tridax procumbens*. Flavonoids are chemical compound which possess anti-oxidant and vasorelaxation properties. It has been shown that they contribute to increase production of nitric oxide (NO) by endothelial cells (Mendes *et al.,* 2011; Si *et al.,* 2014). Their vasorelaxation activity could also involve NO-independent mechanisms. In addition, flavonoids, such as isoquercitrin, could also inhibit angiotensin converting enzyme (ACE) and induce diuretic action (Gasparotto Junior *et al.,* 2011a, b).

Many mechanisms could thus be involved in the antihypertensive effect of *Tridax procumbens*. Firstly, active molecules present in the active fractions could act by decreasing arterial peripheral resistances. Indeed, an important relaxation effect of *Tridax procumbens* aqueous extract on rat aortic

ring has been reported and this vasorelaxation effect was partly NO-dependent (Salahdeen and Murtala 2012; Salahdeen *et al.*, 2012). In view of the hypertension model used, active fractions could thus act directly on arterial vessels by inhibiting L-NAME actions. Secondly, additional mechanism could be a cholinergic effect on heart as it has been shown that the hypotensive effect of *Tridax procumbens* extract was associated with a decrease heart rate, effect which was prevented by atropine administration (Salahdeen *et al.*, 2004). Thirdly, it has been shown that aqueous extract of *Tridax procumbens* induced in salt-loaded rat a decrease in plasma levels of sodium and chloride (Ikewuchi *et al.*, 2010). In addition, as flavonoids were detected in the active fractions and that these compounds have been shown to exert diuretic action, this action mechanism could also be considered.

In conclusion, dichloromethane and ethyl acetate fractions obtained from the crude aqueous extract of the aerial part of *Tridax procumbens* are the fractions which are effective in normalizing arterial blood pressure in L-NAME-induced hypertensive rats. In view of the chemical compounds detected in these fractions, and in comparison with other fractions, the hypertensive activity could be relative to the flavonoid compounds. Further studies must be performed on these fractions in order to identify the antihypertensive molecules and the mechanism of their action.

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