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Evaluation of the hypotensive effect of the aqueous extract of the leaves of *Afrostyrax lepidophyllus* Mildbr (Huaceae) in wistar rats

Wossolo Lingomo B.S.[1, 2,3*], Bafounguila-Ngala S.M[3], Loubanou C.A.C[3], Etou Ossibi A.W[2,3] et Abena A.A[2]

[1.] Biology Department, Faculty of Applied Sciences, DENIS SASSOU-N'GUESSO University, Kintélé, Congo

[2.] Biochemistry and Pharmacology Laboratory, Faculty of Health Sciences, Marien Ngouabi University, P.O. Box 69, Brazzaville, Congo

[3.] Pharmacodynamics and Experimental Pathophysiology Laboratory, Faculty of Science and Technology, Marien Ngouabi University, P.O. Box 69, Brazzaville, Congo

*Corresponding author: Email: *etouarnaud@yahoo.fr*

Abstract

Afrostyrax lepidophyllus Mildbr is a medicinal plant from tropical Africa belonging to the Huaceae family. It is used in traditional medicine for the management of several diseases including high blood pressure. The present work aimed to evaluate the hypotensive effect of the aqueous extract of the leaves of this plant in normotensive wistar rats and to elucidate its probable mechanism of action. The invasive method was used in rats anesthetized with 15% urethane. The results obtained showed that intravenous administration of this extract at doses of 2.5; 5; 10 and 20 mg/kg, bw, iv caused a decrease in mean arterial pressure (MAP) and heart rate (HR) in normotensive rats. This decrease is permanent at a dose of 2.5 mg/kg, bw, iv. At a dose of 2.5 mg/kg, bw, i.v, this extract has an adrenolytic effect (93.59 ± 2.06%). The decrease in MAP caused by this extract at 2.5 mg/kg is partially inhibited by 41.12± 3.07% in rats pretreated with atropine (1 mg/kg, bw, i.v) 5 minutes before. The hypotensive effect of this extract would partially pass through the cholinergic pathway, via muscarinic receptors. This extract also inhibited the action of L-NAME by 39.45± 1.68%; it would also act through the nitric oxide pathway. The hypotensive effect observed in the present study could be due to the presence of alkaloids, anthraquinones, coumarins, flavonoids and tannins contained in this extract.

Keywords: Medicinal plant, *Afrostyrax lepidophyllus*, hypotensive, adrenolytic, cholinomimetic

Introduction

Hypertension is a widespread condition worldwide. It is one of the most serious risk factors for heart, cerebrovascular, and kidney disease and is referred to as the “hidden killer.” It is usually discovered during routine checkups in apparently healthy individuals (WHO, 2023a). Despite advances in modern medicine, the prevalence of this disease continues to rise. The number of hypertensive people worldwide doubled between 1990 and 2019, from 650 million to 1.3 billion, and is expected to reach 1.5 billion by 2025 (WHO, 2023b).Nearly half of people with hypertension worldwide are currently unaware that they have it, and more than three-quarters of adults with hypertension live in low- and middle-income countries (Temgoua et *al.,* 2023; WHO, 2023c). Furthermore, the prevalence of high blood pressure among adults in Africa remains high. In 2019, approximately 35.7% of adults had high blood pressure, compared to 33% of adults in 2010 (Temgoua et *al.,* 2023; WHO, 2023c).Even today, despite advances in pharmaceutical chemistry, the use of medicinal plants for therapeutic purposes is very present in some countries, particularly in developing countries (Wangny et *al.,* 2019). According to the World Health Organization (WHO), nearly 80% of the world's inhabitants use traditional medicines to deal with primary health problems (WHO, 2019).Of the more than 500,000 plant species recorded on the planet, more than 200,000 species found in tropical African countries have medicinal properties (Akka et *al.,* 2016; Wangny et *al.,* 2019). In view of this biodiversity, the WHO recommends that developing countries, on the one hand, initiate programs concerning the identification, preparation, cultivation and conservation of medicinal plants and, on the other hand, evaluate the safety, efficacy and quality of herbal remedies using modern techniques (WHO, 2014). He low purchasing power of African populations and the high cost of treatments push the population to resort to traditional medicine in the treatment of this pathology. Among these plants, there is *Afrostyrax lepidophyllus* Mildbr (*A. lepidophyllus* Mildbr) which, according to some traditional practitioners, would be a "Magic" plant used for the treatment of many conditions including high blood pressure (Bouquet, 1969; Frédéric et *al.,* 2017). Thus, this study is devoted to the research of the hypotensive effect of the aqueous extract of the leaves of *A. lepidophyllus* Mildbr in normotensive wistar rats.

Materials and Methods

Animal Material

Adult male rats, 24 ± 2 weeks old and weighing between 220 and 230 g, were used. These rats were supplied by the animal facility of the Faculty of Science and Technology, where they were kept under standard lighting conditions (12 hours of light, 12 hours of darkness) at a room temperature of 26 ± 1 °C. These rats had free access to standard chow and tap water.

Plant Material

The leaves of *A. lepidophyllus* Mildbr were used in this study. They were collected in Tsiaki in the Bouenza department, 320 km from Brazzaville in the southeast of the Republic of Congo. A leaf sample was identified and compared to a reference specimen registered under No. 2384 in the national herbarium of the National Institute for Research in Exact and Natural Sciences (IRSEN) in Brazzaville, Congo.

Methods

Preparation of the aqueous extract of *A. lepidophyllus* Mildbr leaves

The aqueous extract of *A. lepidophyllus* Mildbr leaves was prepared by a 10% decoction by mixing 100 g of the plant material in 1 L of distilled water. This mixture was boiled for 30 minutes. The resulting decoction was then filtered through Wattman No. 1 paper and concentrated to dryness using a BUCCHII rotary evaporator.

Dose-response effect of aqueous extract of *A. lepidophyllus* Mildbr leaves on mean arterial pressure (MAP) and heart rate (HR)

Animal Preparation

Rats were anesthetized by intraperitoneal injection of 15% urethane at a rate of 1 mL/100 g of rat body weight.

Exposure and catheterization of the femoral vein

The anesthetized rat was fixed in dorsal decubitus using pins planted on its four legs on a cork board. The skin of the inner side of the thigh of the left hind leg was delicately split, allowing the identification in the middle of the femoral space of the pearly red femoral artery, the dark-appearing femoral vein at the back and the femoral nerve. The vein was delicately separated from the artery and cleared for about 1 cm using a suture passer. A ligature was made towards the peripheral end and a waiting suture was passed under the femoral vein. After incision of the vein, a catheter filled with 10% heparinized NaCl was introduced into the vein, we observed a rise in blood in the catheter and then the vein-catheter assembly was firmly ligated. This catheter will be used to administer the products.

Carotid artery exposure and catheterization and measurement of BP and HR

A midline and longitudinal incision of the sternohyoid muscle was made and the trachea was exposed by spreading the muscles. One of the carotids was separated from the nerve fibers and exposed for approximately 1 cm. A ligation on the cephalic side was performed and a holding wire was placed under the artery. A vascular clamp was placed as low as possible towards the heart behind the wire. An incision was made between the first ligature and the holding wire; the free tip of the catheter filled with heparinized NaCl solution (1%) connected to the transducer was introduced into the carotid artery towards the heart and held by the second ligature. When the clamp is removed and the tap connecting the carotid to the transducer is opened, blood flows into the catheter and the transducer transmits the variations in blood pressure to the recorder which converts the waves into a trace that can be viewed on the computer screen (the red band represents the blood pressure, the upper edge of which indicates the systolic and lower edge, the diastolic blood pressure and the green band represents the HR. The data were recorded every five (05) minutes for sixty (60) minutes and the variations in MAP and da HR were calculated by the following formulas: Δ MAP = (Pi – Po) x 100/Pi and (Etou Ossibi et al., 2017). Where :

- Pi = mean arterial pressure at time t after administration of the product
- Po = mean arterial pressure before administration of the product.

Evaluation of the dose-response effect of the aqueous extract of *A. lepidophyllus Mildbr* leaves on MAP and HR in normotensive rats

After the period (1 hour) of stabilization of BP and HR, the aqueous extract of the leaves of *A. lepidophyllus Mildbr* at increasing doses of 2.5; 5; 10; 20 mg/kg, i.v., b.w. was administered to normotensive rats (n = 5) in a single dose. The effects of this extract on BP and HR were observed for one hour after their administration. The dose of 2.5 mg/kg was chosen for the continuation of the work because at this dose, the hypotensive effect persisted for one hour of the experiment.

Study of the probable mechanism of action of the aqueous extract of the leaves of *A. lepidophyllus* Mildbr

Interference aqueous extract of the leaves of *A. lepidophyllus Mildbr* –adrenaline

Two (2) groups of five (5) rats each were formed and treated as follows:

- Group 1 received the adrenaline solution (50 µg/kg, bw, i.v.) alone;
- Group 2 received the aqueous extract of *A. lepidophyllus* leaves (2.5 mg/kg, bw i.v.) then

the adrenaline solution (50 µg/kg, bw i.v.) 5 minutes later.

Interference atropine-aqueous extract of the leaves of *A. lepidophyllus* Mildbr

Two (2) batches of five (5) rats each were formed and treated as follows:

- Group 1 received the aqueous extract of *A. lepidophyllus* leaves (2.5 mg/kg, bw, i.v) alone;
- Group 2 received atropine (1 mg/kg, bw, i.v) then the aqueous extract of *A. lepidophyllus* leaves (2.5 mg/kg, bw, i.v) five (5) minutes later.

Interference aqueous extract of the leaves of *A. lepidophyllus* Mildbr -L-NAME

Two (2) batches of five (5) rats each were formed and treated as follows:

- Group 1 received L-NAME (1 mg/kg, bw, i.v) alone;
- Group 2 received the aqueous extract of *A lepidophyllus* leaves (2.5 mg/kg, bw, i.v) then L-NAME (1 mg/kg, bw, i.v), five (5) minutes later.

Blood pressure changes were noted for one hour after administration of each solution (Ngolo et *al.,* 2018).

Phytochemical Screening

Phytochemical screening was conducted to identify the chemical families present in the aqueous extract of *A. lepidophyllus* Mildbr leaves. This test is based on the formation of insoluble complexes using precipitation reactions or on the formation of colored complexes using coloring reactions (Souley Kallo et al., 2018). Seven (7) chemical families were screened, including alkaloids, anthraquinones, coumarins, flavonoids, mucilages, saponins, and tannins.

Statistical Analysis

Statistical analysis and graphical representation of the data were performed using Excel Office 2013 software. The values expressed in the graphs or tables correspond to the means of a series of values plus or minus the standard error of the mean (M ± SEM). The difference between two values was determined using the Student t-test. The significance threshold was set at $P < 0.05$.

Results

Dose-response effect of aqueous extract of *A. lepidophyllus* Mildbr leaves on MAP in normotensive rats

Figure 1 shows the effect of intravenous administration of the aqueous extract of *A. lepidophyllus* Mildbr leaves on the variation of MAP in normotensive rats. It is clear from this figure that administration of the extract at the respective doses of 2.5; 5; 10 and 20 mg/kg causes an immediate and significant decrease in MAP approximately 5 seconds after its administration. This decrease in MAP is respectively - 20.912 ± 2.46%; - 33.8 11± 4.22% ($p < 0.001$); - 23.156 ± 4.24 and - 25.185 ± 3.94% ($p < 0.01$). This decrease is followed by a non-significant increase (-0.055± 0.000; $p > 0.05$)% at the 5th minute for the dose of 2.5 mg/kg below the initial value. Concerning the doses of 5; 10 and 20 mg/kg the increase exceeds the initial value and is respectively 19.912 ± 1.46%; 10.811± 0.22 ($p > 0.05$)% and 09.821± 0.35 ($p > 0.05$)%. Furthermore, a relapse of PAM was observed from the 30th minute for the 5 mg/kg dose and from the 15th minute for the 2.5 mg/kg dose.

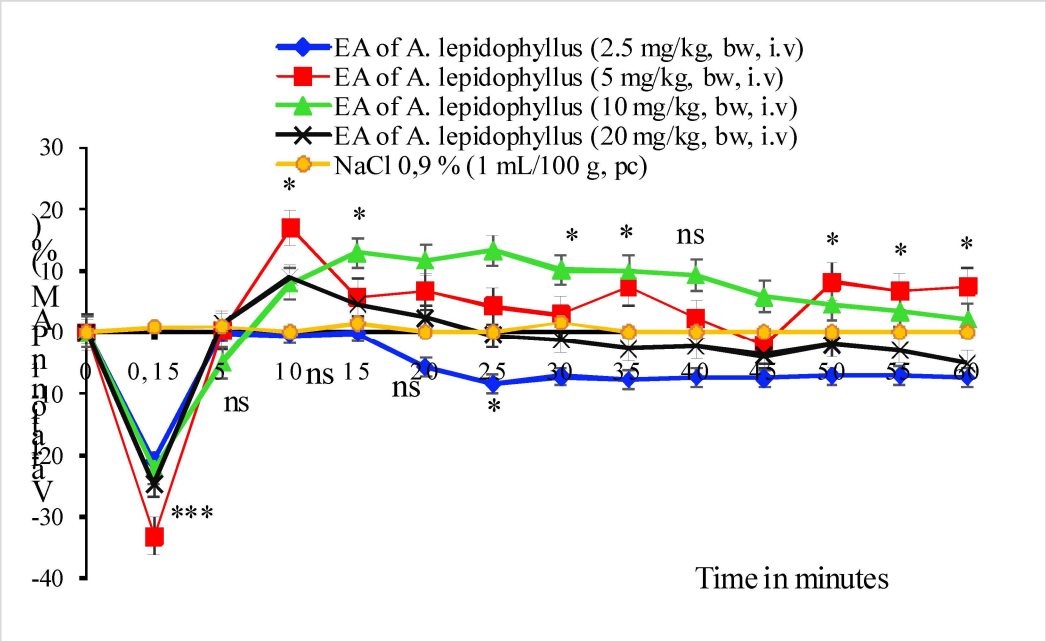

Figure 1: Effect of aqueous extract of *A. lepidophyllus* Mildbr leaves on MAP of normotensive rats. Each point represents mean ± SEM, with n = 5. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: significant difference from baseline MAP and ns: not significant. E.A: Aqueous extract; bw: body weight and i.v: intravenous.

Effect of aqueous extract of *A. lepidophyllus* Mildbr leaves on HR of normotensive rats

Figure 2 shows the effect of intravenous administration of the aqueous extract of *A. lepidophyllus* Mildbr leaves on the variation of HR of normotensive rats. It is evident from this figure that the aqueous extract of *A. lepidophyllus* Mildbr administered at the respective doses of 2.5; 5; 10 and 20 mg/kg causes an immediate and significant decrease in HR after its administration, respectively to - 22.687 ± 2.11% ($p < 0.01$); - 21.593 ± 1.22% ($p < 0.01$); - 20.156 ± 2.24% ($p < 0.01$) and - 33.771 ± 3.94% ($p < 0.001$). This decrease is followed by a non-significant increase (-0.055± 0.000%; $p > 0.05$) at the 5th minute for the dose of 2.5 mg/kg below the initial value followed by a progressive relapse of the MAP from the 10th to the 60th minute. Concerning the doses of 5; 10 and 20 mg/kg the increase exceeds the initial value and is respectively at 19.912 ± 1.46%; 10.811± 0.22 ($p > 0.05$) % and 09.821± 0.35 ($p > 0.05$) %. However, the administration of NaCl 0.9% (1 mL/100 g, bw, iv) did not cause any variation in the rat's HR after 60 minutes of the experiment.

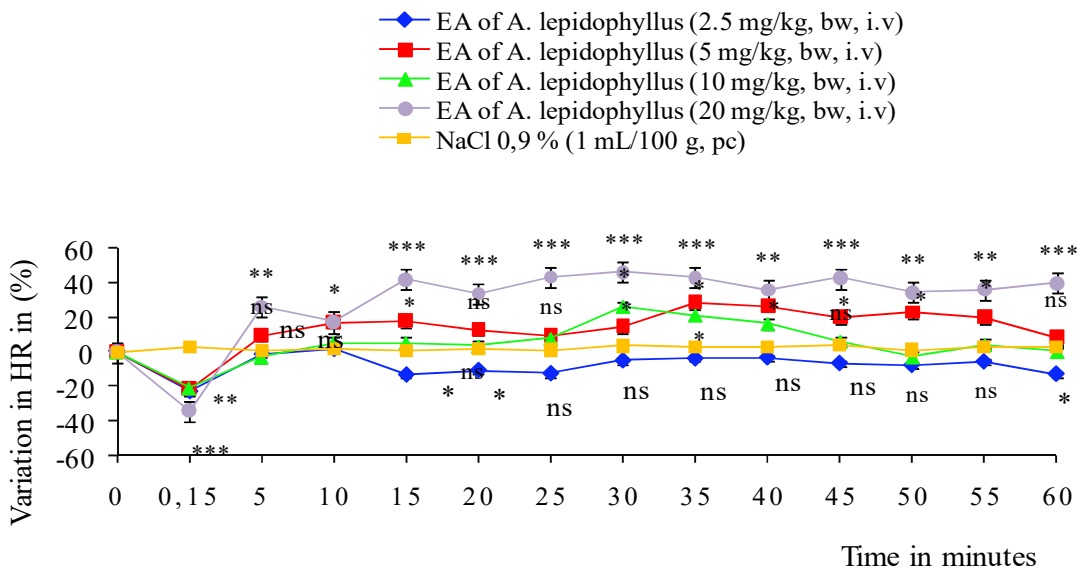

Figure 2: Effect of aqueous extract of *A. lepidophyllus* Mildbr leaves on heart rate in normotensive rats. Each point represents mean ± SEM, with n = 5. * p < 0.05, ** p < 0.01 and *** p < 0.001 significant difference from baseline HR and ns: not significant.E.A: Aqueous extract; bw: body weight and i.v: intravenous and HR: heart rate

Probable mechanism of action of the aqueous extract of the leaves of A. lepidophyllus Mildbr

Effect of aqueous extract of *A. lepidophyllus* Mildbr leaves on adrenaline-induced blood pressure elevation in rats

After intravenous administration of adrenaline (50 µg/kg, i.v) to normotensive rats, MAP immediately increased by 90.02 ± 2.26%, whereas in rats pretreated with the aqueous extract of *A. lepidophyllus* leaves (2.5 mg/kg i.v.), adrenaline administered (50 µg/kg, i.v) five minutes later only caused an increase in MAP of 12.13 ± 0.55%, i.e., an inhibition of 93.59% ± 2.06% (Figure 3).

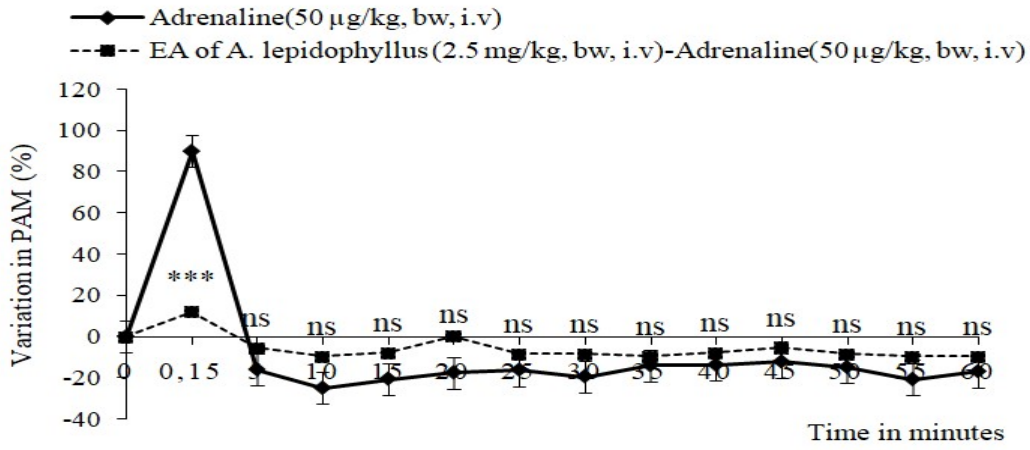

Figure 3: Influence of the aqueous extract of *A. lepidophyllus* Mildbr leaves on adrenaline-induced blood pressure elevation in normotensive rats. Each point is a mean ± SEM, with n = 5 and non-significant difference compared to adrenaline alone values.E.A: Aqueous extract; bw: body weight and i.v: intravenous

Effect of aqueous extract of *A. lepidophyllus* Mildbr leaves on MAP after atropine administration

Figure 4 illustrates the influence of atropine (1 mg/kg i.v) on the effects of the aqueous extract of *A. lepidophyllus* Mildbr leaves (2.5 mg/kg, i.v) in normotensive rats. It follows from this figure that the administration of the aqueous extract of *A. lepidophyllus* leaves (2.5 mg/kg, i.v) in non-atropinized rats causes an immediate decrease in MAP of -21.83 ± 11.72% in normotensive rats compared to -11.23 ± 6.84% ($P < 0.05$) in rats pretreated with atropine (1 mg/kg, i.v) then with the aqueous extract (2.5 mg/kg, i.v); i.e. a percentage inhibition of 41.12%.

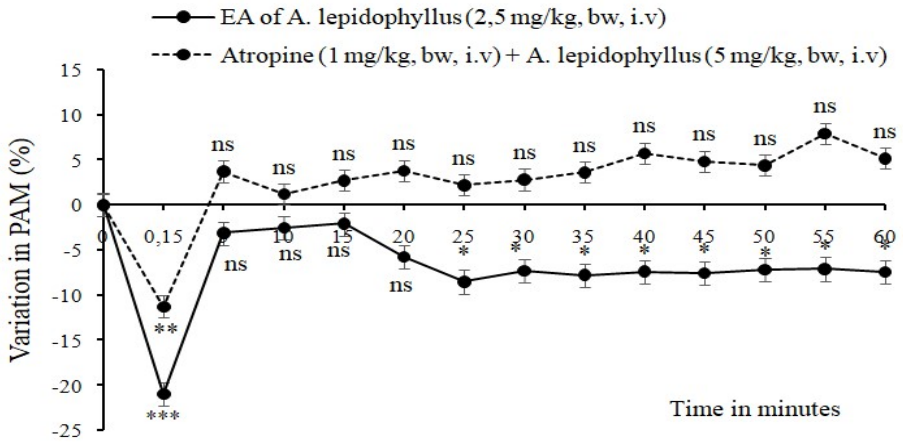

Figure 4: Influence of atropine on the hypotensive effect of the aqueous extract of *A. lepidophyllus* Mildbr leaves in normotensive rats. Each point is a mean ± SEM, with n = 5. * $p < 0.01$ and * $p < 0.001$, significant difference compared to the values of the aqueous extract (2.5 mg/kg) alone.E.A: Aqueous extract; bw: body weight and i.v: intravenous

Effects of *A. lepidophyllus* leaf aqueous extract on L-NAME-induced MAP elevation in normotensive rats

After intravenous administration of L-NAME (1 mg/kg, bw, i.v) in normotensive rats, MAP immediately decreased by -15.36 ± 11.30%, whereas in rats pretreated with the aqueous extract of *A. lepidophyllus*Mildbrleaves (2.5 mg/kg, bw, i.v), L-NAME administered (1 mg/kg, bw i.v) five minutes later only caused a decrease in MAP of -7.30 ± 7.86% ($p < 0.01$), i.e., an inhibition of 39.45 ± 1.68% (Figure 5).

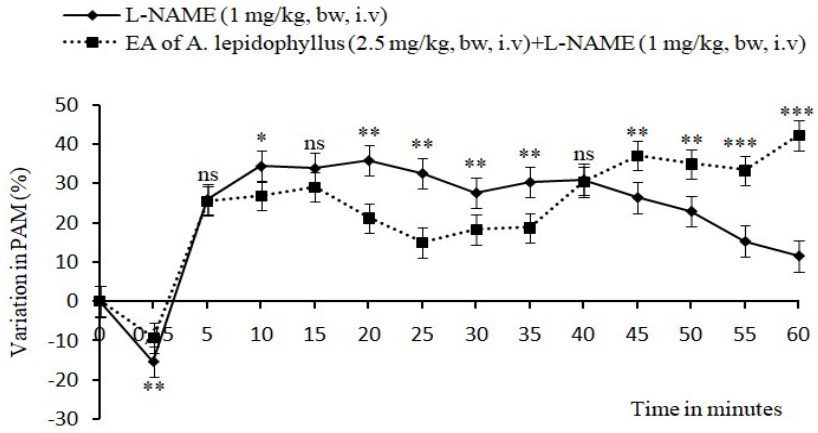

Figure 5: Influence of *A. lepidophyllus*Mildbrleaf aqueous extract on L-NAMEinduced blood pressure elevation in rats. Each point is a mean ± SEM, with n = 5. ** $p < 0.01$ significant difference compared to L-NAME values alone.E.A: Aqueous extract; bw: body weight and i.v: intravenous

Phytochemical composition of the aqueous extract of *A. lepidophyllus* leaves

Table I shows the different phytochemical families contained in the aqueous extract of *A. lepidophyllus* Mildbr leaves. It is clear from this table that the aqueous extract of *A. lepidophyllus*Mildbr leaves contains alkaloids, anthraquinones, coumarins, flavonoids and tannins. On the other hand, mucilages and saponins are absent.

Table I: Phytochemical constituents of *A. lepidophyllus* leaves

Chemical families	Results
alkaloids	+
Anthraquinones	+
Flavonoids	+
Mucilages	-
Coumarins	+
Sapononins	-
Tanins	+

+: presence and -: absence

Discussion

The present study aimed to evaluate the hypotensive activity of the aqueous extract of *A. lepidophyllus* Mildbr leaves, as well as to elucidate its probable mechanism of action. To this end, the hypotensive effect of the aqueous extract of *A. lepidophyllus* Mildbr leaves was evaluated using the invasive method in normotensive rats. The results obtained show that intravenous administration of the aqueous extract of *A. lepidophyllus* Mildbr leaves causes an immediate and significant drop in mean arterial pressure (MAP) in normotensive rats. This extract therefore causes hypotension which could be explained by its action on the contractile activity of the heart and/or on peripheral vascular resistance (Tom et al., 2011; Etou Ossibi et al., 2014).The decrease in MAP caused by this extract is followed by a rapid increase above the initial value for doses of 5, 10 and 20 mg/kg on the one hand and on the other hand this rise remains below the initial value for the dose of 2.5 mg/kg. At doses of 2.5 and 5 mg/kg, this observed hypotensive effect is maintained for up to one hour of experimentation. The rapid increase in MAP observed could be explained by a reflex phenomenon following the increase in the discharge of catecholamines (Aboubakar et al., 2012; Etou Ossibi et al., 2017).Indeed, the drop in systemic blood pressure causes, in the short term, the secretion of noradrenaline and adrenaline following the stimulation of the sympathetic nervous system and the adrenal medulla respectively. These two substances pass into the blood and cause stimulation of the heart and vasoconstriction of the blood vessels, which causes the rise in blood pressure (Etou Ossibi et al., 2017; Ngolo et al., 2018).However, the persistent hypotensive effect observed with doses of 2.5 and 5 mg/kg could well be explained by a decrease in peripheral vascular resistance or by an increase in the production of NO and prostacyclin secreted by the vascular endothelium, having a powerful vasodilatory effect on smooth muscle cells (Etou Ossibi et al., 2017; Fauzy et al., 2019).Furthermore, intravenous administration of the aqueous extract to normotensive rats results in a significant decrease in heart rate, and this decrease is more significant at a dose of 2.5 mg/kg. The observed decrease in HR could partly explain the decrease in MAP observed in this present study. These results suggest that the aqueous extract of *A. lepidophyllus* Mildbr leaves contains bioactive substances capable of lowering heart rate, in particular polyphenols, coumarins and tannins (Frédéric et al., 2017; Mboungou-Bouesse et al., 2022).The effects observed

(decrease in MAP and HR) with this extract are not dose dependent because at the lowest dose (2.5 mg/kg), a significant decrease in MAP and HR was observed, maintained below the initial value for one hour of observation compared to the other doses (5, 10 and 20 mg/kg, bw, i.v).It is in this context that the dose of 2.5 mg/kg is chosen and used for the study of the mechanism of action in order to know by which route this extract would pass to reduce the blood pressure and heart rate observed above.For this purpose, adrenaline (50 µg/kg, bw i.v) was administered alone to normotensive rats to ensure its hypertensive effect. The results obtained show an increase in the mean arterial pressure (90.02± 2.26%) of normotensive rats compared to the initial arterial pressure. This increase confirms the hypertensive effect of the adrenaline used (Ngolo et al., 2018).On the other hand, adrenaline (50 µg/kg, i.v.) administered 5 minutes after the administration of the aqueous extract of *A. Lepidophyllus* Mildbr (2.5 mg/kg, bw, i.v.) only causes an increase in mean arterial pressure of 12.13 ± 0.55%, i.e. an almost total inhibition of 93.59 ± 2.06%. This result suggests that there would be an antagonism between the extract and adrenaline. The aqueous extract of the leaves of *A. lepidophyllus* Mildbr would therefore have an adrenolytic effect. This result suggests that this extract could therefore have antihypertensive properties. Indeed, it is known that adrenaline causes an increase in blood pressure by increasing cardiac output following stimulation of the myocardium and by constricting peripheral vessels following an increase in calcium influx (Etou Ossibi et al., 2014).The fact that this extract inhibited the rise in blood pressure induced by adrenaline, we can say that the hypotensive effect of this extract could be partly explained by the reduction in calcium influx that it would cause by activating the muscarinic acetylcholine receptors (Etou Ossibi et al., 2014).To verify this hypothesis, a study of the influence of atropine on the hypotensive effect of the aqueous extract of *A. lepidophyllus* Mildbr was evaluated in normotensive rats. The results obtained show that atropine (1 mg/kg, bw, i.v) administered 5 minutes before the aqueous extract of the leaves of *A. lepidophyllus* Mildbr(2.5 mg/kg, bw, i.v) causes an inhibition of the hypotensive effect of the latter, i.e. a percentage of inhibition of 41.12± 3.07%. This result confirms the hypothesis that this extract would act in part on muscarinic acetylcholine receptors. Atropine is known to be an anticholinergic, it competitively opposes muscarinic acetylcholine receptors. This inhibition manifests itself on the heart by blocking the excitation of the vagus nerve. Regularly, the vagus nerve releases acetylcholine, which slows the heart rate. By limiting this activity, atropine anticipates the transmission of acetylcholine to its receptors, thus decreasing parasympathetic tone and increasing heart rate, which results in an increase in blood pressure (Belemnaba et al., 2021).The partial inhibition of the hypotensive effect of the aqueous extract of *A. lepidophyllus* Mildbr leaves (2.5 mg/kg, bw, i.v) by atropine would probably be explained by this mechanism and suggests that this extract would therefore contain cholinomimetic or parasympathomimetic substances which would mimic the effect of acetylcholine on its receptors. In order to verify the hypothesis that the observed relapse of PAM would be due to the decrease in peripheral vascular resistance. An interference between the aqueous extract of the leaves of *A. lepidophyllus* Mildbr (2.5 mg/kg, bw, i.v) and L-NAME (1 mg/kg, bw, i.v) was carried out in normotensive rats. The results obtained show that L-NAME (1 mg/kg, bw, i.v) administered 5 minutes after the aqueous extract of the leaves of *A. lepidophyllus* Mildbr (2.5 mg/kg, bw, i.v) causes an inhibition of the hypotensive effect of the latter, i.e. a percentage of inhibition of 39.45±1.68%.L-NAME is known to cause increased blood pressure through vascular stiffness due to decreased bioavailability of nitric oxide (NO) due to inhibition of nitric oxide synthase (NOS) (Belemnaba et al., 2021).Since the aqueous extract of *A. lepidophyllus* Mildbr leaves (2.5 mg/kg i.v.) inhibited the action of L-NAME, we can suggest that this extract would promote the production of NO, which is a powerful vasodilator responsible for the decrease in peripheral vascular resistance and therefore the subsequent decrease in mean arterial pressure. The vasorelaxation responsible for the hypotensive effect of this extract would involve

the nitric oxide pathway. The different effects observed in the present study could be justified by the presence of alkaloids, anthraquinones, coumarins, flavonoids and tannins contained in this extract. These substances, in particular alkaloids, coumarins and flavonoids, are recognized for their hypotensive, vasorelaxant and bradycardic effects (Simko et al., 2018).

Conclusion

The present work aimed to evaluate the hypotensive effect of the aqueous extract of the leaves of *A. lepidophyllus* Mildbr in the wistar rat. The results obtained show that this extract has a hypotensive effect which is permanent at the dose of 2.5 mg/kg, bw, iv. At the dose of 2.5 mg/kg, bw, this extract opposes the elevation of the mean arterial pressure induced by adrenaline (50 µg/kg, bw, i.v). At this same dose, this hypotensive effect is partially inhibited by atropine (1 mg/kg, bw, i.v) and L-NAME at (1 mg/kg, bw, i.v). The hypotensive effect observed in the present study could be justified by the presence of alkaloids, anthraquinones, coumarins, flavonoids and tannins contained in this extract.

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