Anxiolytic activity of aqueous extract from the trunk bark of *Pausinystalia yohimbe* (Rubiaceae) K. Schum in wistar rats.

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Keywords: *Pausinystalia yohimbe,* Traction, forced swimming, Anxiolytic

Submitted 22/03/2024, Published online on 05/06/2024 in the *Journal of Animal and Plant Sciences (J. Anim. Plant Sci.) ISSN 2071 – 7024*

1 SUMMARY

The objective of this study is to evaluate the effects of aqueous extract from the trunk bark of *Pausinystalia yohimbe* (Rubiaceae) k. schum on the traction assay in Swiss mice and forced swimming in Wistar rats. The traction test was carried out according to the method of Julou and Courvoisier. Twenty-five mice were divided into five batches each containing five animals as follows: batch (1) control, the animals received distilled water (0.5ml/kg P.O), batch (2) where the animals received Diazepam (3mg/kg P.O). In batches 3, 4 and 5 the animals received the aqueous extract of the trunk bark of *Pausinystalia yohimbe* (100, 250 and 500 mg/kg P.O). The immobility time of the animals on the bar was recorded every five minutes for fifteen minutes. The study of forced swimming was carried out according to the method described by Porsol, like the previous study, the same number of animals also divided into five batches. With a batch of reference molecule namely Clomipramine (15mg/kg P.O) and the batches where the animals were treated at doses of (100; 250 and 500mg/kg P.O) of the aqueous extract of the trunk bark of *Pausinystalia yohimbe*. The parameters evaluated were swimming time, climbing time and immobility time for six minutes... It appeared from this study that the aqueous extract of the trunk bark of *Pausinystalia yohimbe* significantly increased ($P<0.01$; $P<0.001$) the immobility time. The aqueous extract would act like Diazepam hence has sedative properties. The aqueous extract would act like Clomipramine and has antidepressant properties. The aqueous extract of the trunk bark of *Pausinystalia yohimbe* would be a good pharmacological product to recommend for mental pathologies.

2 INTRODUCTION

Scourges such as unemployment, poverty, pathologies are characteristic elements of human life in our various societies. They create worries in both men and women that cause mental illnesses such as depression. Depression is a pathological state in which an individual suffers psychologically with a pessimistic questioning of himself and the environment (Goetz, 2018). The World Health Organization (WHO 2013a) predicts that unipolar depression will be the most debilitating disease in the coming years (Andlin *et al.*, 2005). Depression is therefore a priority issue for health and social policy, with depressive disorders affecting one in five people in their lifetime, with an average onset of 30 years of age according to current research findings (Baer *et al.*, 2013). Several psychotropic drugs have been introduced specifically as sedatives and hypnotics. The sedative effect leads to a decrease in motor activity and a

decrease in alertness, which causes sleep induction. Thus, the use of medicinal plants is now a safe and effective avenue for a better well-being of populations. *Pausinystalia yohimbe*, a species found in the great forests of Central Africa (Adjanohoun, 1988), has been traditionally known since. *Pausinystalia yohimbe* is used in the Congolese pharmacopoeia for the treatment of many pathologies, including sexual impotence (Akassa *et al.*, 2022). In addition to sexual impotence, some populations that use *Pausinystalia yohimbe* report sedative and depressive effects. It was in this spirit that we became interested in this plant. These properties being raised, the main question to be resolved in this study remains that of the effective therapeutic dose to relieve.

3 MATERIALS AND METHODS

3.1 Plant material: The plant material used consists of the trunk bark of *Pausinystalia yohimbe*. Vendors at the Total market in Brazzaville (Akassa et al., 2022) provided the bark. The identification of *Pausinystalia yohimbe* was made at the National Institute for Research in Exact and Natural Sciences (I.R.S.E.N.) and compared with the reference sample from the Central Herbarium registered under the number 15694-2009.

3.2 Animal Material: Three (3) month old albino rats of the wistar strain and weighing between 162 and 205g and albino mice of the Swiss strain weighing between 20 and 25g were used. They were raised at the animal facility of the Ecole Normale Supérieure (ENS) of the Marien Ngouabi University and fed with a standard diet with free access to water, and a night-day lighting rhythm (12/12).

3.3 Methods

3.3.1 Preparation of the aqueous extract: Fifty (50) grams of powder from the trunk bark of *Pausinystalia yohimbe* were dissolved in 500 ml of distilled water. The mixture is macerated under a magnetic stirrer (model L-73) for 48 hours (Akassa *et al.*, 2022). The maceration obtained is filtered using filter paper (Standard Pleats or hydrophilic cotton). The filtrate obtained was concentrated on a water bath thermostatically controlled at 55°C, which made it possible to obtain 3.5 g of marro-coloured dry extract.

3.4 Study of the effect of *Pausinystalia yohimbe* aqueous extract on traction in mice

3.4.1 Study of psychotropic activity : This work was initiated to evaluate the sedative effect of the aqueous extract of the trunk bark of *Pausinystalia yohimbe* on the tensile test.

3.4.2 Tensile Testing: In this test, mice are suspended by their forelegs from a metal wire stretched horizontally. 15 minutes after administration of the aqueous extract from the trunk bark of *Pausinystalia yohimbe*, the mouse is grasped by the skin of the back and tail, the thread is presented to their forelegs, and the mouse is released as soon as the legs have grasped the thread. Twenty-five (25) mice were divided into five (5) lots, each containing five (5) animals, and treated Group 1 (control) animals were given distilled water at a dose of 0.5 ml/100 g PO; Group 2 animals received Diazepam (Valium) at a dose of 3mg/kg PO. Groups 3; 4 and 5, animals were given aqueous extract from the trunk bark of *Pausinystalia yohimbe* (100; 250 and 500 mg/kg PO). Observations were made repeatedly for three times within a five (5) minute interval. The parameter studied was:

The behaviour of mice at the fixed bar "the animal

Group 1 (control) was given distilled water at a dose of 0.5 ml/100 g PO;

Group 2 received clomipramine at a dose of 15 mg/kg PO;

Groups 3; 4 and 5 received the aqueous extract from the trunk bark of *Pausinystalia yohimbe* (100; 250 and 500 mg/kg PO). Thirty minutes after administration of the aqueous extract from the trunk bark of *Pausinystalia yohimbe* and Clomipramine, the animals are placed in turn in a jar containing water; the observation is made for six minutes.

4 RESULTS

4.1 Effect of *Pausinystalia yohimbe* aqueous trunk extract on traction in Swiss mice.

4.1.1 Tensile Testing: The aqueous extract of the trunk bark of *Pausinystalia yohimbe* (100; 250 and 500 mg/kg Per-os) administered in mice caused an increase (P<0.001) in the immobility time of the mouse at the bar compared to animals given distilled water (0.5 ml/kg Person-os). This increase ranges from 3.70 ± 1.04 to 4.42 ± 2.76 for aqueous extract from (Table 1).

Table 1: Effects of aqueous extract from the trunk bark of *Pausinystalia yohimbe* K. Schum on the tensile test in mice as a function of time.

Time(Second)	Distilled water (0.5ml/kg)	Diazepam (3mg/kg)	P. Yohimbe (100mg/kg)	P. Yohimbe (250mg/kg)	P. Yohimbe (500mg/kg)
] 0 – 5]	2.01 ± 0.08	3.14 ± 0.67 ***	2.54 ± 0.37 ***	3.70 ± 1.05 **	2.33 ± 0.48 ***
] 5 – 10]	2.02 ± 0.99	3.45 ± 1.07 ***	2.19 ± 0.59 ***	3.03 ± 0.76 ***	2.17 ± 0.46 ***
] 10 – 15]	2.11 ± 0.49	3.57 ± 0.90 ***	2.50 ± 0.21 ***	4.42 ± 2.76 **	2.02 ± 0.30 ***

** p< 0,01; *** p < 0,001; (n=5)

The results are expressed as an average ± scartypes; n=5 mice per batch; P<0.001: Highly significant difference compared to control mice.

4.2 Effect of the aqueous trunk extract *Pausinystalia yohimbe* on the forced swimming test in the Wistar rat: The aqueous extract of the trunk bark of *Pausinystalia yohimbe* at doses (100; 250 and 500 mg/kg) administered to rats increased (p< 0.05; p<0.001) swimming time in rats compared to animals administered to distilled water at a dose of 0.5ml/100g body weight (Figure 1). This increase is from 98.8; 119.4; 146.2; in animals that have received the aqueous extract from the bark.

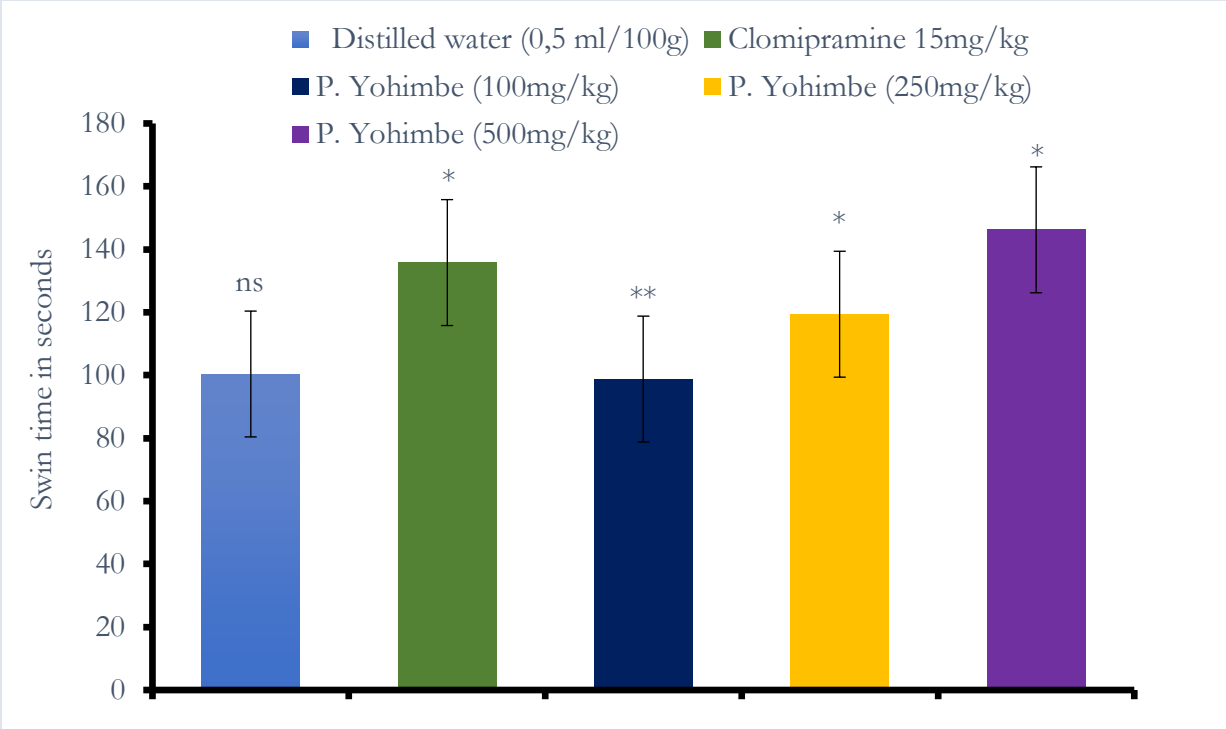

Figure 1: Swimming time as a function of treatments in animals
Values are expressed as an average ± scartypes n=5 rats per lot *P<0.05; **P<0.001: significant and very significant difference compared to control rats.

4.3 Escalation Time: Aqueous extract from the trunk bark of *Pausinystalia yohimbe* at doses (100, 250 and 500 mg/kg) administered to rats increased ($p< 0.05$; $p<0.001$) the climbing time compared to animals given distilled water (0.5 mg/100 g body weight) (Figure 2). An increase in climbing time was observed in rats treated with aqueous extract of the trunk bark of *Pausinystalia yohimbe* at a dose of 250mg/kg. This increase goes from; 108.8; 123.8; 144.4 in animal.

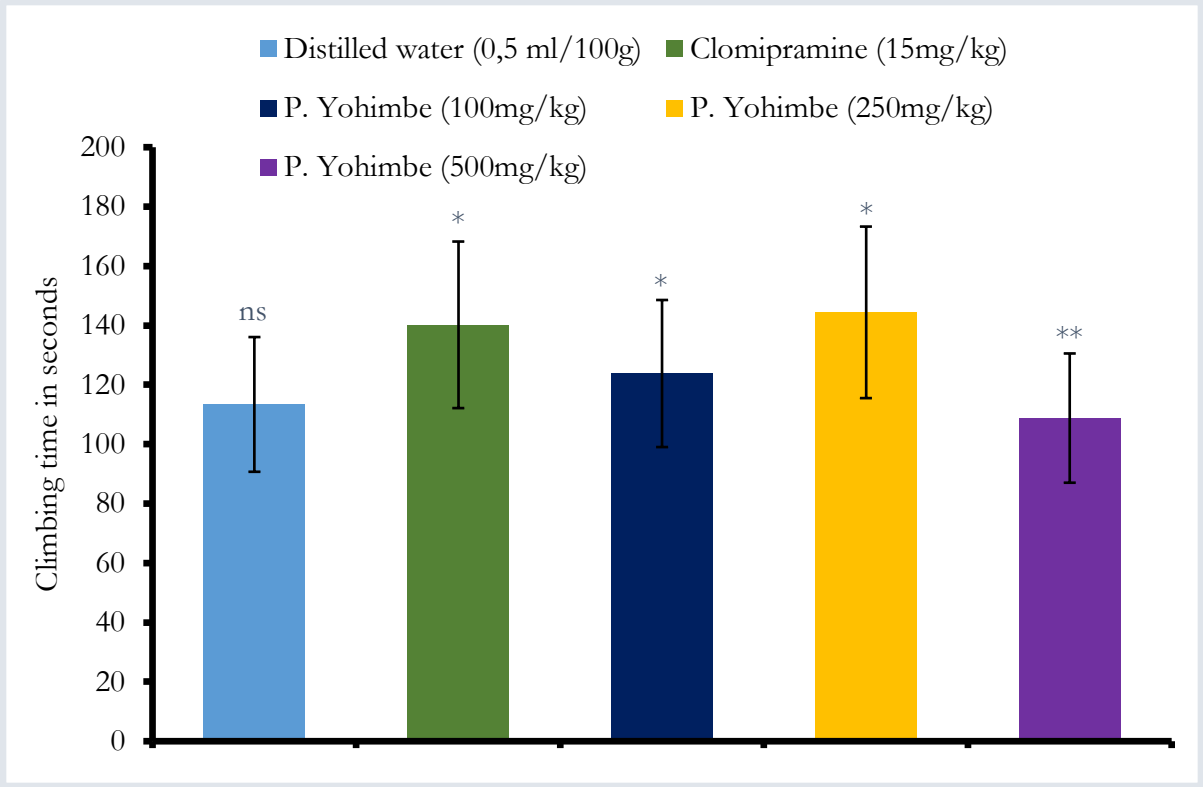

Figure 2: Escalation times as a function of treatments in animals
Values are expressed as mean ± SD n=5 rats per batch *P<0.05; **P<0.001: significant and highly significant difference from control rats.

4.4 Immobility time: The aqueous extract of *Pausinystalia yohimbe* trunk bark at doses (100, 250 and 500mg/kg) administered to rats reduced (p<0.05) immobility time compared with animals administered distilled water (0.5ml/100g body weight) (figure 3). This increase was 43.8; 88.6; 106.4; in rats administered aqueous extract of *Pausinystalia yohimbe* trunk bark (500; 100 and 250mg/kg respectively) and 45.8 versus 146.2 in the control group.

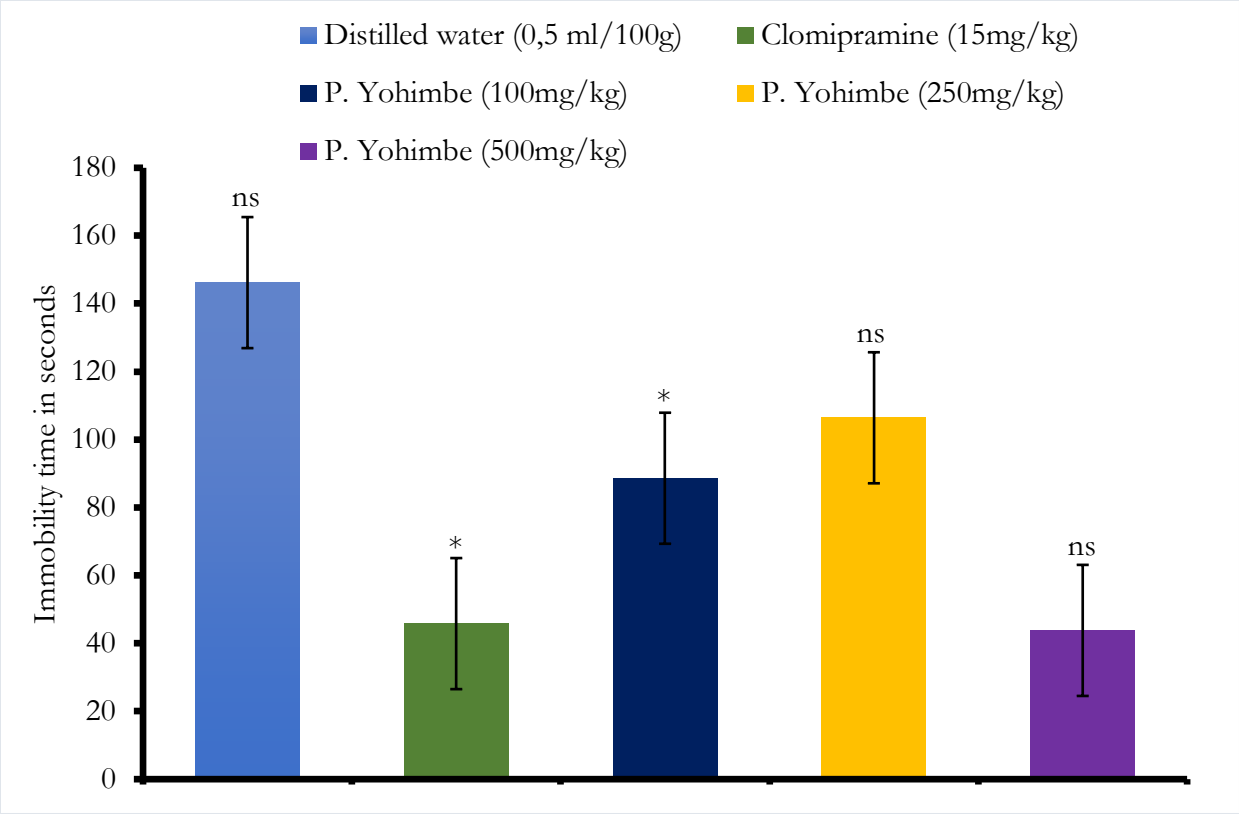

Figure 3: Immobility times as a function of treatments in animals
Values are expressed as mean ± SD n=5 rats per batch *P<0.05: significant difference from control rats

5 DISCUSSION

The aim of this study was to evaluate the effects of aqueous extract of *Pausinystalia yohimbe* trunk bark in the treatment of depression in wistar rats. The results obtained show that the aqueous extract of *Pausinystalia yohimbe* trunk bark (100, 250 and 500mg/kg PO) increases the animal's immobility time at the bar compared with animals given distilled water (0.5ml/100g PO). This can be explained by the animal's recent fatigue. *Pausinystalia yohimbe* therefore has a sedative effect, acting like Diazepam. Diazepam potentiates the inhibitory effect of gamma-aminobutyric acid (GABA) in the central nervous system (Hajjaj, 2017). GABA is the main neurotransmitter inhibiting neuronal functions; it can be identified as an "endogenous anxiolytic". These results are similar to those found by NENE (2010), who worked on evaluating the effect of an aqueous extract of *Bridelia ferruginea* Benth on spontaneous and locomotor activity in rats. These results are also similar to those found by Hajjaj, (2017) on the aqueous extract and essential oils of *Matricaria chamomila. L* and *Ormenis mixta.L.* This study also assessed the antidepressant effect using the forced-swimming test induced by aqueous extract of *Pausinystalia yohimbe* trunk bark in albino Wistar rats. Administration of the aqueous extract of *Pausinystalia yohimbe* trunk barks (100; 250 and 500mg/kg) causes an increase ($P<0.05$; $P<0.001$) in swimming time, climbing time and a decrease ($P<0.05$) in immobility time compared with animals given distilled water (0.5ml/100g body weight). This indicates that the animal is in a depressed state. These results suggest that the aqueous extract of *Pausinystalia yohimbe* trunk bark acts as an antidepressant (Clomipramine), the reference molecule used in this study. Clomipramine is an antidepressant that acts as a non-selective inhibitor of serotonin and noradrenaline reuptake Chantale (2006). These results are

similar to those of Rachedi Bachir' (2009), who showed that administration of Ketoconazole to rats during forced swimming also increased swimming time, climbing time and decreased immobility time. These results are also similar to those of (Rex al.2004), who demonstrated that during the forced swimming test, rats show active behaviour represented by increased swimming and climbing time, and passive behaviour represented by a decrease in immobility time. (Hajjaj 2017). It should be noted that depression involves a central serotonin deficiency. It can therefore be said that the aqueous extract of *Pausinystalia yohimbe* trunk bark contains substances that help increase serotonin levels. The literature shows that alkaloids have an inhibitory effect on monoamine oxidase, inhibiting the reuptake of dopamine and serotonin (Hajjaj *et al.*, 2017). Previous chemical profiling studies reveal the presence of alkaloids in the trunk bark of *Pausinystalia yohimbe* Akassa (2019). The aqueous extract of this plant therefore has antidepressant properties. Yaser Rahmati, *et al.*, 2013 also obtained the same results with in a study evaluating the antidepressant effect of the aqueous and hydroalcoholic extract of the essential oils of *Matricaria chamomila. L* and *Ormenis mixtal.* in the animal model of depression, using fluoxetine, an inhibitor of serotonin reuptake by the transporter (Sert) present on serotonin neurons, as the reference molecule (Hajjaj, 2017). It should be noted that depression involves a deficit in serotonin at the central level. on the evaluation of the antidepressant effect in animal models of depression in humans, and which used as reference molecule, fluoxetine, an inhibitor of the reuptake of serotonin by the transporter (Sert) present on serotonin neurons (Hajjaj, 2017). We therefore understand that depression involves a central serotonin deficiency. Thus, our results may be explained by the activity of the alkaloids contained in the aqueous extract, which appear to have an inhibitory effect on monoamine oxidase by inhibiting dopamine and serotonin reuptake (Hajjaj, 2017). Animals treated with the aqueous extract of *Pausinystalia yohimbe* trunk bark show an increase in swimming time. A previous study shows that during the forced swim test, antidepressants producing predominant noradrenergic or dopaminergic elevations reduce immobility by increasing climbing time. On the other hand, antidepressants that activate serotonin (5-HT) reduce immobility by increasing swimming time (Wilks *et al.*, 2000). In summary, despite the limitations of the animal model used, our results suggest that the aqueous extract of *Pausinystalia yohimbe* trunk bark appears to have sedative and antidepressant properties.

6 CONCLUSION AND OUTLOOK

The study evaluating the effect of aqueous extract of *Pausinystalia yohimbe* trunk bark on anxiolytic activity in rats and mice shows: Aqueous extract of *Pausinystalia Yohimbe* K. trunk bark. Schum (100; 250 and 500mg/kg P.O.) has sedative properties in mice, and antidepressant properties in rats. The mode of action of the aqueous extract of *Pausinystalia yohimbe* therefore remains to be investigated in future studies.

7 ACKNOWLEDGEMENTS

The authors would like to thank all the technicians of the two laboratories in which this study was carried out. Particular thanks are due to Mr. BOKATOLA Philon Martial for his availability and for looking after the animals in the laboratory.

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