The Pharmaceutical and Chemical Journal, 2019, 6(5):45-57

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Sedative and Anxiolytic Properties of Aqueous Extract of Seeds of *Picralima Nitida* (Apocynaceae)

Georgy Djounda D[1], Rigobert Ayissi M E*[2], Antoine Kandeda K[3], Guessong[1], Josaphat Ndelo D[4], Théophile Dimo[3]

[1]Higher Institute of Health Sciences, Universite des Montagnes, Bangangte, Cameroun
[2]Departments Biological Sciences, Higher Teacher's Training College, University of Yaounde I, Yaounde, Cameroun
[3]Department of Animal Biology and Physiology, Faculty of Sciences, University of Yaounde I
[4]Department of pharmacy, Faculty of Pharmacy, University of Kinshasa
*Correspondance to espybass@yahoo.fr

Abstract This study aimed to assess the anxiolytic and sedative properties and influence on biochemical stress markers of aqueous extract of seeds of *Picralima nitida staph* . The effects of three doses (25, 50 and 90 mg/kg) administered *per os* have been compared to distilled water as negative control and 5 mg/kg (or 30 mg/kg) of intraperitoneal diazepam as positive control for anxiety test (or sedation test). Firstly the extract was screened to identify the major active compounds. Then behavioral tests including Elevated Plus-Maze (EPM), Open Field (OF), and Hole-board (HB) were used to assess the level of anxiety in different treatments, and potentiation of diazepam-induced sleep test for sedation, Forced Swim Test (FST) to induce chronic stress. After FST, markers of oxidative stress were measured. Results indicate that the extract of *P. nitida* possesses alkaloids, saponins, flavonoids, tannins, and polyphenols. The EPM-test indicates that at all the doses used, *P. nitida* induced an increase of open arms time. In the OF-test, *P. nitida* at doses 25 and 50mg/kg significantly increased the number of crossing as well as central time. In the HB-test, the extract induced an increase of head-dip number and duration. Extract at 25mg/kg increased the duration of diazepam-induced sleep. Biochemical studies have shown an increase of gamma aminobutyric acid (GABA) and serotonin level, an increase of reduced glutathione (GSH) and superoxide dismutase (SOD) activities in the brain and a decrease of malondialdehyde (MDA). These results indicate that P. *nitida* can be used as anxiolytic and antioxidant agent.

Keywords stress, anxiety, neurotransmitters, oxidative stress, acute stress, chronical stress

Introduction

Anxiety can be defined as an emotional, reactive, generalized state [1], characterized by increasing probabilities of triggering emotional reactions similar to those of fear [2], in response to an unknown danger, threats, frustration or failure, situations of novelty or uncertainty [3]. Chisholm and colleagues reported an increase of 50% of mental disorders between 1990 and 2013 in 36 countries [4]. Therefore, it is a public health issue since countries worldwide need healthy citizens to realize development. Though there is a lack of available data on mental disorders in Africa and especially in Cameroon, the population are used to face and overcome these conditions. In human medical sciences, several medicines are used in the treatment of these conditions, including selective serotonin reuptake

inhibitors and benzodiazepines for anxiety. The intake of these medicines is often associated with side effects after a long run. Traditional medicines have a significant interest especially in Africa and Asia, where about 80% of the population preferentially use traditional medicines for primary health care [5]. Several conditions, including anxiety and mental disorders, are hence treated using medicinal plants. Many of these plants have been found to be efficient in the treatment of anxiety namely; *Chenopodium ambrosioides*, *Mimosa pudica*, *Microglosa pyriflora* [6], *Passiflora edulis* [7]. *Picralima nitida* is used in many countries for many purposes, especially as anti-malaria, treating digestive diseases like diarrhea, typhoid, as stimulant, and bracing [8,9,10]. The stimulant properties of *P. nitida* retained our attention. This lead us to assume its properties on the central nerveous system. Here we assessed anxiolytic and sedative properties of *P. nitida*, its antioxidant and stress neuromediators level influence to verify these assumptions.

Materials and Methods

Plant and preparation of extracts

The Fruits of *P. nitida* were provided from the forests of Mbam Division in the Center Region, and the plant identified in the National Herbarium of Cameroon in Yaounde under reference number 23183/HNC. Seeds of *P. nitida* after being dried in the stove were pulverized, and 365mg of powder was extracted in 5 liters of hot distilled water (infusion), after filtration on Whatman paper No 3, the filtrate was dried in the stove for 3 days and 52g of dried aqueous extract obtained, resulting in an extraction yield of 14.42%.

Phytochemical screening

The presence of alkaloids was assessed by Dragendorf's reactant combined with Meyer's reactant, saponins by foam height measurement after boiling, flavonoids using Shinoda test, Tannins by 2% ferric chloride and Stiany reactant, triterpenes and sterols by Lieberman-Buchard test, polyphenols with 2% Ferric chloride reaction, anthocyanins by concentrated Sulfuric Acid/Ammonia, reducing sugars by Fehling test, coumarins using sodium hydroxide reactant, and quinones by Borntraeger reactant. The screening was done as previously described by Bokota's team [11], Kouakou and colleagues [12].

Behavioral studies

Animals

Albino *Mus musculus* swiss mice of both sexes aged between 2 and 3 months, weighing from 20 to 30 g, breded at the Animal House of the Faculty of Science of the University of Yaounde I were used. They had free access to water and food. Animal handling was done in accordance with the National Ethics Guide FWA-IRB00001954, and all the substances were administered in a volume of 10 ml/Kg.

Assessment of anxiolytic properties of P. nitida seeds aqueous extract

30 mice were randomly divided into 5 groups (n=6) according to sexes and mass. Group I received distilled water per os. Group II was treated with 5 mg/kg of intraperitoneal Diazepam. Groups III, IV, and V orally received doses 25, 50, 90 mg/kg of aqueous extract of seed of *P. nitida*. 1 hour (or 30 minutes, depending administration route) after the treatment, animals were submitted to EPM, OF, and HB paradigms.

Potentiation of Diazepam induced sedation

24 mice were randomly divided into 4 groups (n=6) according to sex and mass. Group I received 30 mg/kg of intraperitoneal Diazepam. Groups II, III, and IV were treated by 30 mg/kg of intraperitoneal Diazepam, followed 30 min later by oral administration of, 25, 50 and 90 mg/kg of aqueous extract respectively and the total sleeping time measured.

Forced swim test

36 mice were randomly divided into 6 groups (n=6) according to sex and mass. Group I as normal control orally received distilled water. Group II as negative control orally received distilled water. Group III was treated by 5mg/kg of intraperitoneal diazepam as a positive control. Groups IV, V and VI were treated respectively with 25, 50 and 90 mg/kg of oral administered aqueous extract. Except for Group I, 1 hour after treatments, individually each animal was submitted to FST for a cut off time of 300 sec. For 10 consecutive days, swimming duration and immobility time were measured. At day 10, animals were submitted to EPM.

Biochemical measurements

At day 11, mice were sacrificed, brains collected and divided into 2 and crushed separately in ceramic mortar. The first moiety underwent homogenization in 2 ml of Tris-HCl buffer (50mM, pH=7.4). The second moiety homogenized in 2ml of methanol and both moieties were centrifuged at 4000 rpm for 25 min. In the methanolic homogenate, the brain levels of serotonin, GABA and GABA-T were measured. In Tris-HCl homogenate, SOD, glutathione and malondialdehyde brain levels were dosed on spectrophotometer Thermo Fisher brand.

Data analysis

All data were express as mean ± SEM. Comparison between groups was performed using one-way ANOVA test, followed by Turkey post-Test. A p-value of less than 0.05 was considered to be significant

Results

Phytochemical screening

The qualitative phytochemical screening identifies different types of a secondary compound which are present in the aqueous extract as shown in table 1.

Table 1: Phytochemical qualitative screening of aqueous extract of *P. nitida*

Screened compounds	Test used	Results
Alkaloids	Dragendorf and Meyer	**positive**
Polyphenols	2% Ferric chloride	**positive**
Coumarins	Sodium hydroxide	negative
Anthocyanins	Concentrated Sulfuric Acid/Ammonia	negative
Flavonoids	Shinoda Test	**positive**
Saponins	Foam height measurement (>1Cm)	**positive**
Reducing sugar	Fehling Test	negative
Gallic tanins	2% Ferric chloride	negative
Catechic tanins	Stiany reactant	**positive**
Quinones	Borntraeger reactant	negative
Sterols	Lieberman-Buchard Test	negative

Anxiolytic effects of *P. nitida* on acute stress

Elevated Plus Maze

We observed a significant increase regardless of dose, of TSO in groups treated with extract at doses 25, 50 and 90 mg/Kg compared to the negative control group. 5mg/kg of intraperitoneal diazepam caused an increase of the TSO compared to the negative control. Dose 25mg/kg has induced a 27.74% reduction in TSC and diazepam, a 22.74% reduction of TSC.

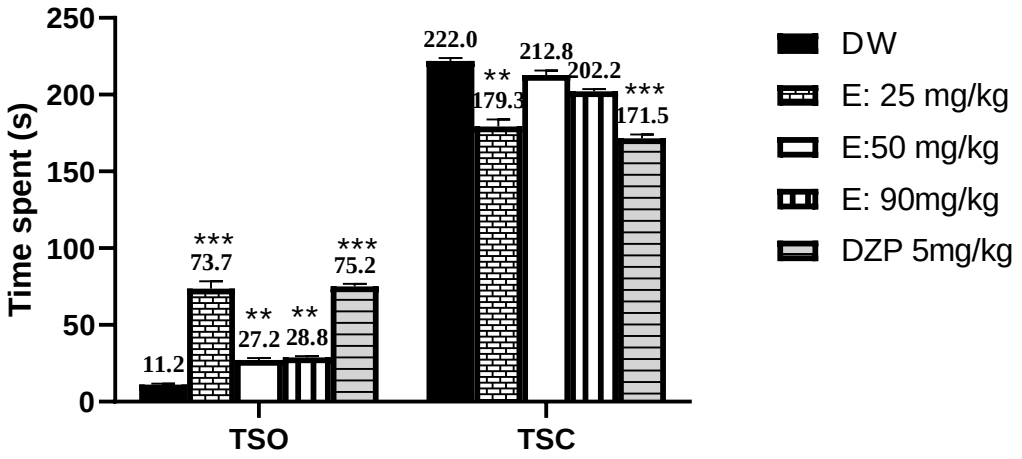


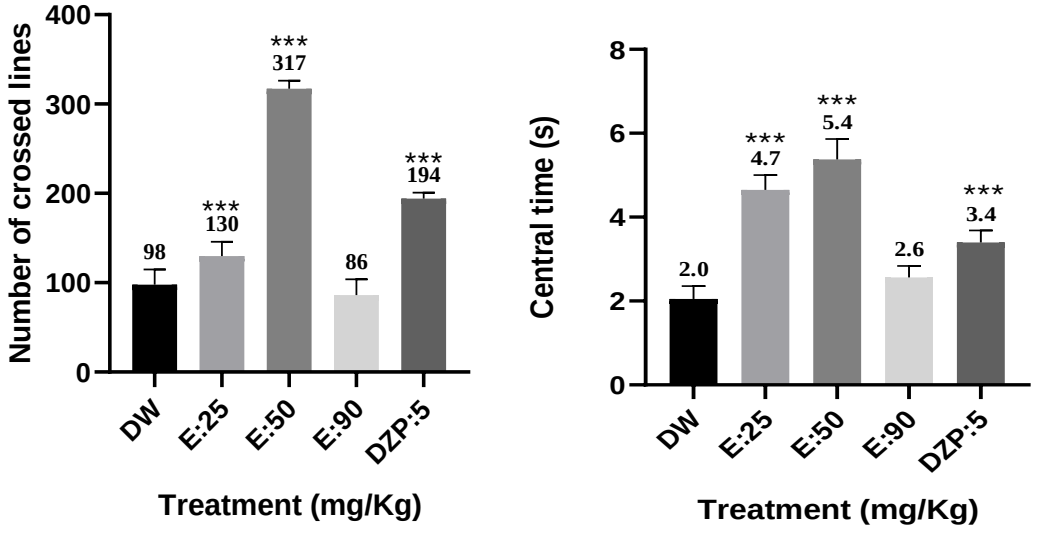
Figure 1: Effects of P. nitida on acute stress in EPM test

Data were expressed as mean + SEM (n=6). Differences were significant for P< 0.05 (*), P< 0.0011(**) and P<0.0002 (***) when compared to negative control group.

TSO: Time spent in open arms (s), TSC: Time spent in closed arms (s), DW: Distilled Water (negative control), DZP: diazepam (positive control), E: Aqueous extract of *P. nitida*

Open Field (OF)

Doses 25 and 50 mg/kg have significantly increased the number of crossing from 97.83+7.00 in the negative control to 129.70 ± 6.65 and 317.00 ±3.72 respectively and the central time from 2.04 ± 0.12 sec in the negative control to 4.65±0.14 sec and 5.37 ± 0.19 sec respectively. Diazepam significantly increased the crossing number by 98% and the central time by 64% compared to the negative control group. It was also observed a decrease of 82.5% and 50% of stool production at doses 25 and 50mg/kg of aqueous extract respectively when compared to the negative control. Diazepam caused a 75% reduction in stool production compared with the negative control.



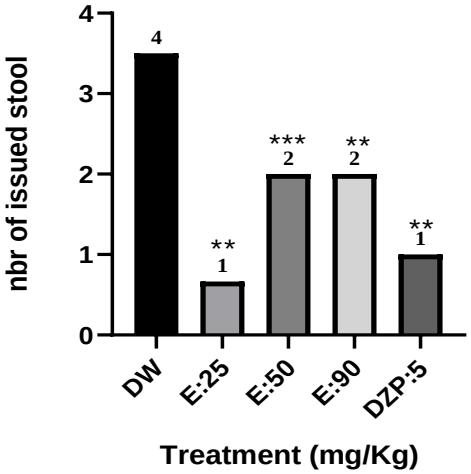


***Figure 2**: Effects of P. nitida on Open Field parameters*

Data were expressed as mean + SEM (n=6). Differences were significant for P < 0.05 (*), P<0.0011(**) and P<0.0002 (***) when compared to negative control group.

DW: Distilled Water (negative control), DZP: diazepam (positive control), E: Aqueous extract of *P. nitida.*

Hole Board (HB)

Head dip increased from 1.83 ± 0,40s in the negative control to 7.20 ± 0.47s, 8.20 ± 0.70s, and 15.17 ± 1.01s at doses 25, 50 and 90 mg/kg respectively. Number of head dip switched from 14.00 ± 0.33 to 25.00 ± 1.08 (dose 25mg/kg), 28.00 ± 3.87 (dose 50 mg/kg) and 39.00 ± 5.91 (dose 90 mg/kg). Diazepam has significantly increased the number of head-dip by 46% a head dip duration by 90% compared to the negative control.

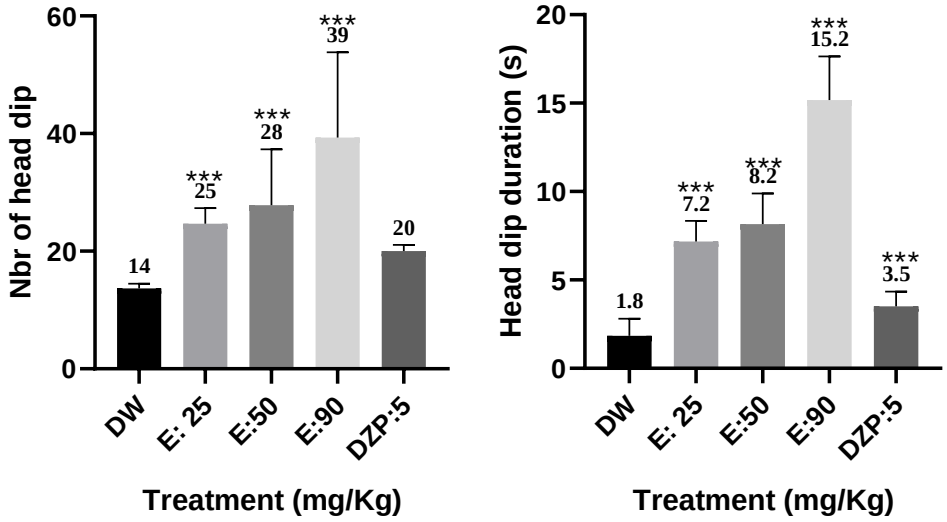


***Figure 3**: Effects of P. nitida in the evolution of Hole Board parameters*

Data were expressed as mean + SEM (n=6). Differences were significant for P < 0.05 (*), P<0.0011(**) and P<0.0002 (***) when compared to negative control group.

DW: Distilled Water (negative control), DZP: diazepam (positive control), E: Aqueous extract of *P. nitida.*

Sedative properties of *P. nitida*

Results show a significant 23% increase in the sleep duration at dose 25mg/kg compared with positive control and the increase of the sleep latency at the same dose of extract induced an 89% increase of latency.

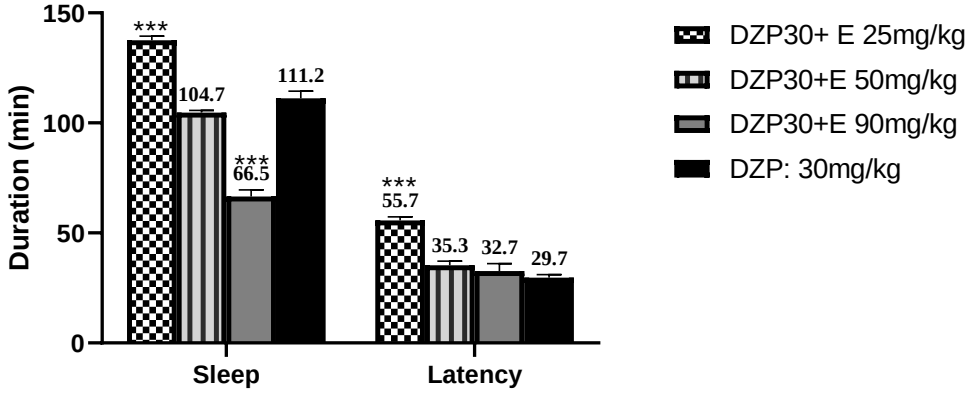


***Figure 4**: Effect of P. nitida on diazepzm-induced sleep duration*

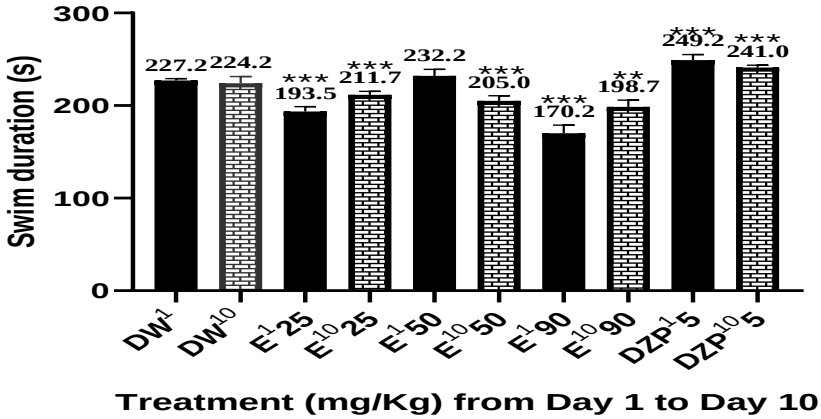
Data were expressed as mean + SEM (n=6). Differences were significant for P < 0.05 (*), P<0.0011(**) and P<0.0002 (***) when compared to diazepam treated group.

DZP: diazepam (positive control), E: Aqueous extract of *P. nitida*

Curative effect of *P. nitida* on the sub-chronically induced stress by the forced swim test

Swim duration: We observed that on Day1, at doses 25 and 90 mg/kg, a significant decrease of swim time by 14.75% and 25.03% respectively compared to the negative control group. After 10 days of treatment, doses of 25, 50 and 90 mg/kg of *P. nitida* extract decreased swimming time by 5.67%, 8.64%, and 11.46% compared to the negative control. Diazepam (5 mg/kg, PI) increased swimming time on the first day by 9.76% compared to the negative control and by 7.39% after 10 days of treatment, respectively. Between day1 and day10, doses 25 and 90 mg/kg increased by 9.83% and 16.75% respectively.

Immobility time: The immobility time decreased from 73.00 ± 0.75 sec in the negative control on Day 1, to 75.60 ± 2.96 sec at Day 10. On day1, the extract at doses 25 and 90mg / kg increased by 45.89% and 77.85% the immobility time compared to the negative control. On day 10, the 3 doses (25, 50 and 90 mg/kg) increased this duration by 16.84%, 25.66%, and 34.03% compared to the negative control group; between day 1 and day 10 the noticed decrease was 17.05%, and 21.95% for the doses 25 and 90 mg/kg compared to the first day.



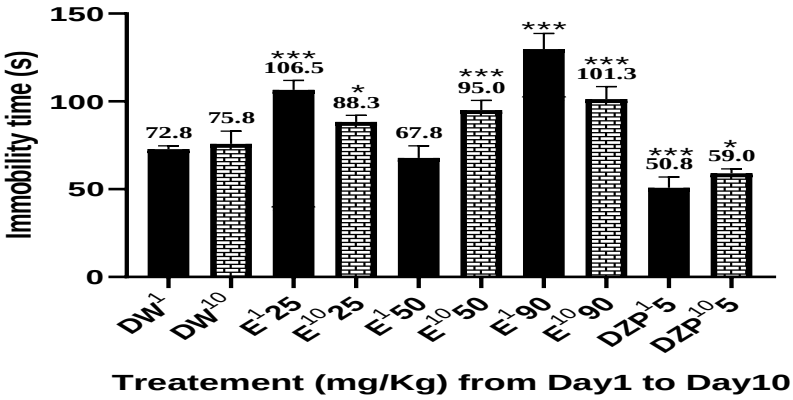


Figure 5: Effects of P. nitida on forced swim induced stress

Data were expressed as mean + SEM (n=6). Differences were significant for P < 0.05 (*), P<0.0011(**) and P<0.0002 (***) when compared to the negative control group.

DW: Distilled Water (negative control), DZP: diazepam (positive control), E: Aqueous extract of P. nitida

Effect of *P. nitida* on EPM parameters after the sub-chronically induced stress by the forced swim

The data indicate in "prolonged treatment + sustained stress" an increase by doses of 25mg/kg, 50 mg/kg and 90 mg/kg in a dose-dependent manner, of the TSO compared to the negative control. The extract at doses 25, 50 and 90 mg/kg decreased significantly and dose-dependently the TSC by 4.75%, 9.3%, and 15.23% respectively.

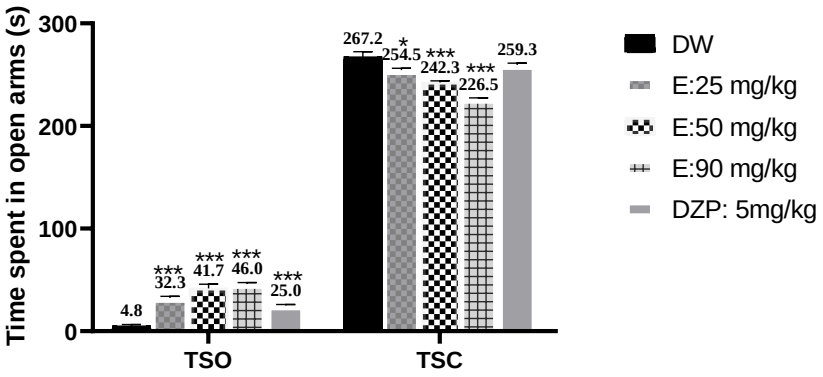


Figure 6: Effects of P. nitida on EPM parameters after 10 days treatment

Data were expressed as mean +/- SEM (n=6). Differences were significant for P < 0.05 (*), P<0.0011(**) and P<0.0002 (***) when compared to the negative control group.

DW: Distilled Water (negative control), DZP: diazepam (positive control), E: Aqueous extract of *P. nitida.*

Biochemical Measurement

Neurotransmitters

1) GABA and GABA-T

The brain GABA level in the normal control group (NS) is 2.00 ± 0.36 µg/ml, similar to those of the positive control group (2.00 ± 0.20 µg/ml. This brain level of GABA increased from 0.81 ± 0.07 µg/ml in the negative control group to 1.80 ± 0.06 µg/ml, 2.9 ± 0.05 µg/ml and 2.1 ± 0.16 µg/ml at doses 25, 50 and 90 mg/kg respectively.

The brain level of GABA-T in the normal control group is $6.00 \times 10^{-6} \pm 1.71 \times 10^{-7}$ pg/min/mg of organ and $3.50 \times 10^{-5} \pm 1.00 \times 10^{-6}$ pg/min/mg of organ in the negative control group. The extract at doses 25 and 50 mg/kg respectively

significantly decreased by 85% and 40% the activity of GABA-T compared to the negative control group. The activity of GABA-T was 4.00 x 10^{-5} ± 5.65 x 10^{-7} pg/min/mg in the positive control group.

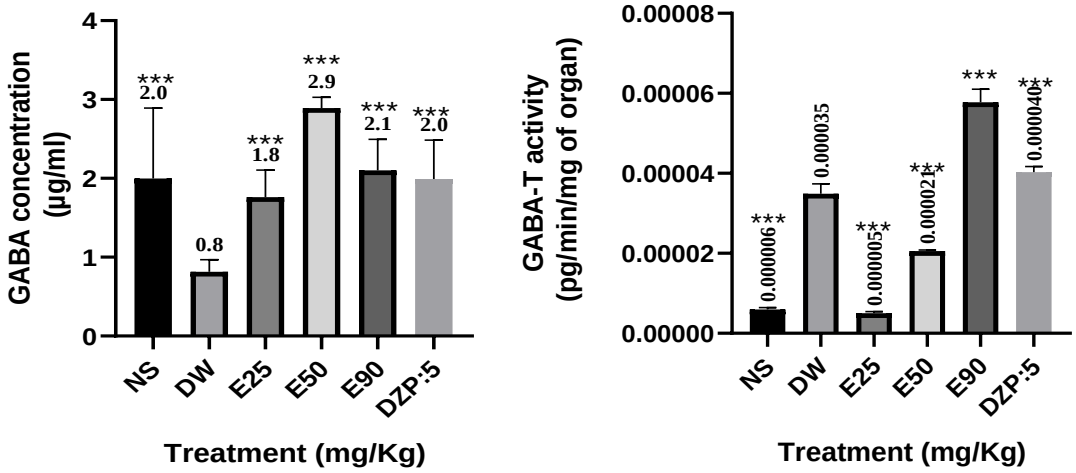


Figure 7: Influence of P. nitida on brain level of GABA and GABA-T

Data were expressed as mean +/- SEM (n=6). Differences were significant for P<0.05 (*), P<0.0011(**) and P<0.0002 (***) when compared to the negative control group.

NS: Not stressed (normal control); DW: Distilled Water (negative control), DZP: diazepam (positive control), E: Aqueous extract of *P. nitida.*

2) SEROTONIN

Figure 8 shows that the concentration of serotonin decreased from 15.10 ± 0.54 ng/ml in the normal control (NS) to 6.40 ± 0.52 ng/ml in the negative control. The 10 days administration of three doses of *P. nitida* seeds aqueous extract, as well as diazepam, resulted in a significant increase in the concentration of serotonin in the brain of mice when compared to the negative control group.

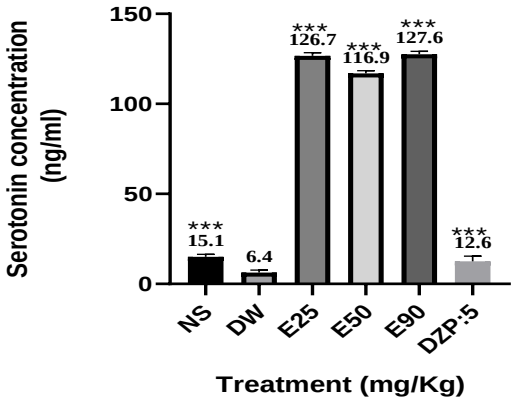


Figure 8: Effects of P. nitida on serotonin brain level

Data were expressed as mean +/- SEM (n=6). Differences were significant for P < 0.05 (*), P<0.0011(**) and P<0.0002 (***) when compared to the negative control group.

NS: Not stressed (normal control); DW: Distilled Water (negative control), DZP: diazepam (positive control), E: Aqueous extract of *P. nitida.*.

Stress enzyme markers

1) SOD

SOD activity increased from 17.30 ± 0.90 units/mg in the normal control (NS) group to 4.90 ± 0.18 units/mg of organ in the negative control group. The aqueous extract of *P. nitida* significantly increased the activity of SOD by 78.64% at dose 25 mg/kg and by 21.16% at dose 90 mg/kg, compared to the negative control group. Administration of diazepam also resulted in a decreased SOD activity compared to the negative control group.

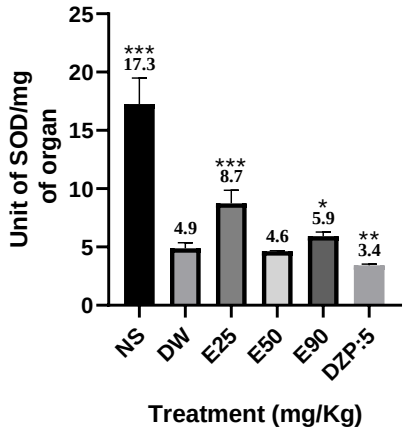


Figure 9: Effects of P. nitida on superoxide dismutase activity

Data were expressed as mean +/- SEM (n=6). Differences were significant for P< 0.05 (*), P<0.0011 (**) and P<0.0002 (***) when compared to the negative control group.

NS: Not stressed (normal control); DW: Distilled Water (negative control), DZP: diazepam (positive control), E: Aqueous extract of *P. nitida*.

2) **GSH**

Figure 10 shows a decrease of glutathione level from 8.6 x 10^{-5} ± 5.13 x 10^{-6} mM/g of organ in the normal control group (NS) to 2.52 x 10^{-5} ± 9.09 x 10^{-7} mM/g of organ in the negative control. Administration of the aqueous extract and submission to forced swimming for10 consecutive days resulted in a significant (P <0.0002) increase in reduced glutathione (GSH) levels at doses 25, 50 and 90 mg/kg. Diazepam also significantly increased the level of GSH compared to the negative control group.

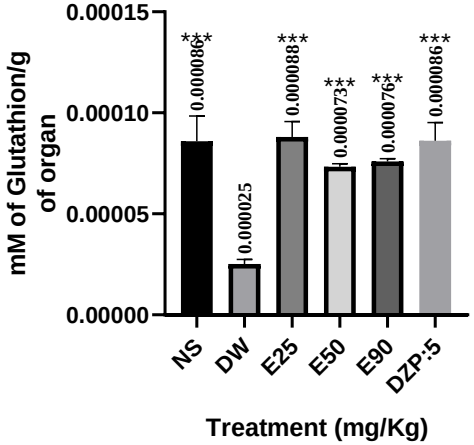


Figure 10: Effects of P. nitida on glutathione level

Data were expressed as mean +/- SEM (n=6). Differences were significant for P< 0.05 (*), P<0.0011 (**) and P<0.0002 (***) when compared to the negative control group.
NS: Not stressed (normal control); DW: Distilled Water (negative control), DZP: diazepam (positive control), E: Aqueous extract of *P. nitida.*

3) MAD

The results indicate that administration of dose 50 mg/kg of extract of *P. nitida*, as well as diazepam (5 mg/kg), resulted in a decrease of MDA level compared to the negative control. The MDA level decreased by 26.32% in the extract-treated mice at dose 50 mg/kg and by 34.75% in the diazepam-treated mice.

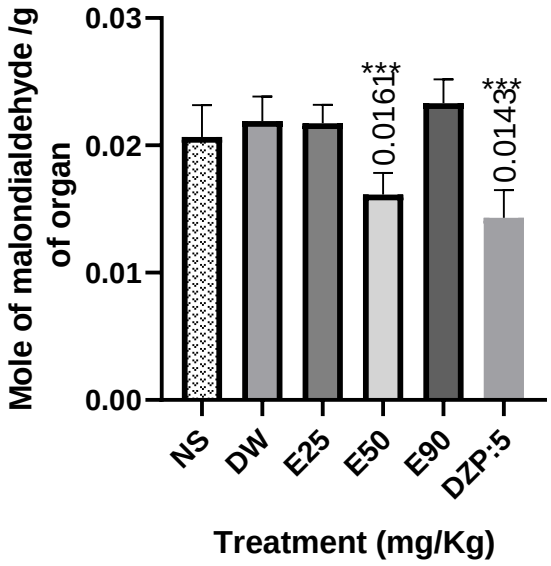


Figure 11: Effects of P. nitida on malondialdehyde

Data were expressed as mean +/- SEM (n=6). Differences were significant for P< 0.05 (*), P<0.0011(**) and P<0.0002 (***) when compared to the negative control group.
NS: Not stressed (normal control); DW: Distilled Water (negative control), DZP: diazepam (positive control), E: Aqueous extract of *P. nitida.*

Discussion

The aim of the present study was to evaluate the anxiolytic and sedative activities of the aqueous extract of seeds of *P. nitida* in mice. The initial qualitative phytochemical screening showed the presence in the extract of alkaloids, catechin tannins, saponins, polyphenols, and flavonoids. These results are in agreement with the work of Bokota's team [11].

The EPM test has traditionally been used to evaluate the effects of anxiolytic and anxiogenic drugs [13]. In the present work, the treatment of mice with diazepam, a well-known anxiolytic, resulted in a significant increase of open arm exploration. With the three doses of the aqueous extract of seeds of *P. nitida* used, the difference in the closed arms exploration was significant only at dose 25 mg/kg. The anxiolytic compounds, by decreasing anxiety, increase the open arms exploration time as well as the number of open arm entries [13]. According to Chintawar and colleagues in 2002, EPM is also used to evaluate memory and learning processes in rodents [14]. Assuming from the author's work that learning reflects the prolongation in latency of transfer from the closed arm to the open arms, the mice treated with our extract would have retained the aversive quality of the open arms. This result can be explained by admitting that the saponins contained in the extract increase memorization in mice [15]. Following long term-

administration, anxiolytic effects were more noticeable than effects on memory. These results are consistent with those of Lister in 1987 [13].

The paradigm of the OF is a very popular animal model that evaluates anxiolytic activity [16]. Subjected to such situation, rodents spontaneously prefer the periphery of the device to an activity in the central part of the apparatus. Rats and mice move closer to the walls and this behavior is so called thigmotaxis. The results of this study showed that the aqueous extract of *P. nitida* seeds increased the number of lines crossed and the duration in the central area of the paradigm while decreasing the number of stools issued. The increase of the time spent in the central zone, the locomotion and the decrease in the number of stools are indicators of the anxiolysis [17].

The HB test is a simple method to measure the animal's response to an unfamiliar environment and is widely used to assess emotionality, anxiety, and/or animal stress response [18]. In this test, the head-dipping behavior is sensitive to the change in the emotional state of the mice and suggests that the expression of anxiolysis in these animals would result in an increase in head-dipping number and duration. The extract of *P. nitida* significantly increased in the dose-related manner the diving (head dip) duration and number. These results suggest that *P. nitida* contains secondary metabolites with anxiolytic activity [19].

The aqueous extract increased the duration of sleep induced by diazepam and increased sleep latency at low dose (25 mg/kg). High doses (50 and 90 mg/kg), on the other hand, reduced the duration of sleep. This would be due to the presence in this extract of metabolites having different effects on the same receptors. The involvement of a larger number of central receptors at high doses can, in this case, induce opposite effects. Indeed, the studies of Menzies and colleagues reported the presence in the seeds of *P. nitida* of alkaloids such as akuammidine, akuammine, akuammicine, akuammigine and pseudoakuammigine which have an opioid activity by acting on mu, kappa,and delta receptors; and that depending on the targeted receptors would have opposite effects. Akuammine is a mu-opioid receptor antagonist whereas in the same extract, akuammicine is a mu-receptor antagonist.The mild sedative activity observed in this study may result from the action of alkaloids demonstrated during phytochemical screening [20].

After 10 days of forced swimming, in the FS test it was observed a decrease of the swimming time and an increase in the duration of immobility in mice treated with the aqueous extract of *P. nitida*. These results are different from those of Walf in 2004, according to which anxiolytic/antidepressant substances increase the duration of swimming and getaway trial while reducing the time of immobility [21]. The extract, however increased between day1 and day10 the duration of swimming and reduces the initial duration of immobility.

Results of biochemical assays showed that the extract corrected the decrease observed in the negative control group compared to the normal control, the level of GABA and serotonin. Doses 25 and 50 mg/kg of *P. nitida* corrected the increase observed in the negative control group compared to the normal control, of the activity of GABA-T. GABA-T (or GABA transaminase) is a GABA degrading enzyme, the increase of this enzyme could suggested the persistent anxiety. The decrease in GABA-T activity observed at low doses confirms the anxiolytic activity of low doses of *P. nitida*.

The increase of serotonin concentrations would also demonstrate anxiolytic activity [22], the extract may contain secondary metabolites acting as selective serotonin reuptake inhibitor (SSRI). SSRIs are able to induce an increase in GABA concentration [23]. The increase in GABA concentration observed in our study is related to anxiolysis due to the aqueous extract of *P. nitida*. Since anxiety may lead to oxidative stress, anxiolysis may also lead to a reduction in oxidative stress. In this way, enzymatic assays showing the effect of aqueous extract of *P. nitida* on stress markers are important. The increase in the activities of the superoxide dismutase enzymes, reduced glutathione and the decrease in the level of MDA connote a strengthening by the extract of the anti-free radical protective systems and thereby an antioxidant activity of extract of *P. nitida*. This result is consistent with the work of Issa in 2016 [24]. The qualitative phytochemical screening of extract of *P. nitida* has revealed among others, the presence of flavonoids and saponins, probabbly responsible of the antioxidant power of this plant [25, 26]. Diazepam throughout the experiment and especially in chronic administration, shows at several points results contrary to those expected especially in EPM in chronic (an increase of TSC instead of a decrease), in the dosages of GABA-T (increase instead of decrease), SOD (decrease instead of increase). This is explained by the fact that in this

long run administration of diazepam, a tolerance to Diazepam would have raised and which prevents its anxiolytic and sedative action in the long term. The work of Divljaković J et *al* [27] shows that administration of diazepam at doses greater than or equal to 2 mg / kg for 14 days resulted in tolerance to anxiolytic diazepam.

Conclusion

The objective of our study was to assess the anxiolytic and sedative properties of the aqueous extract of *P. nitida* seeds by determination via qualitative phytochemical screening of major secondary metabolites, the determination of anxiolytic activity in acute and chronic stress, the sedative properties of the extract by its potentiation on sleep induced by diazepam and the evaluation of the effects of the extract on some biochemical stress markers. It appears that our extract contains among others alkaloids, saponins, tannins, polyphenols, and flavonoids. The aqueous extract of *P.nitida* has anxiolytic properties on the mouse in acute stress and during chronic stress. The aqueous extract of *P.nitida* showed sedative properties at the lowest dose used in this study. The aqueous extract of *P. nitida* improves the biochemical parameters by its anxiolytic and antioxidant potential. This result makes it possible to say that *P. nitida* can, therefore, be used as an anxiolytic drug and as an antioxidant drug.

References

[1]. Kitchem, H., Aronson, AL., Bittle, JL., Mcpherson, CW., Morton, DB., Pakes, SP., et al. (1987). Panel report on the colloquium on recognition and alleviation of animal pain and distress. *Journal of the American Veterinary Medical Association*, 191 (10): 1186-1191.

[2]. Pageat P. (1998). Pathologie du comportement du chien. 2nd ed. Maisons-Alfort : *Les Editions du Point Vétérinaire*, 382 p.

[3]. Chaurand, JP. (1987). L'anxiété en clinique féline. *Le Point Vétérinaire*, 19 (108): 497-502.

[4]. Chisholm, D., Sweeny, K., Sheehan, P., Rasmussen, B., Smit, F., Cuijpers, P., et al. (2016). Scaling-up Treatment of Depression and Anxiety: a global return on investment analysis. *Lancet Psychiatry*, 3(5):415-24.

[5]. OMS | Lancement par l'OMS de la première stratégie mondiale pour les médecines traditionnelles ou parallèles [Internet]. WHO. [consulted 2019 Aug 21]. Available from: https://www.who.int/mediacentre/news/releases/release38/fr/

[6]. Ngo Bum, E., Soudi, S., Ayissi Mbomo, RE., Dong, C., Lakoulo, H., Seke Etet, PF., et al. (2011). Anxiolytic Activity Evaluation of Four Medicinal Plants from Cameroon. *African Journal of Traditional, Complementary, and Alternative Medicines*, 8(5):130-9.

[7]. Petry, RD., Reginatto, F., De-Paris, F., Gosmann, G., Salgueiro, JB., Quevedo, J., et al. (2001). Comparative pharmacological study of hydroethanol extracts of Passiflora alata and Passiflora edulis leaves†. *Phytotherapy Research*, 15(2):162-4.

[8]. Wosu, LO., Ibe, CC. (1989). Use of extracts of Picralima nitida bark in the treatment of experimental trypanosomiasis: A preliminary study. *Journal of Ethnopharmacology*, 25(3):263-8.

[9]. Laure, B., Penlap, V., Kouam, J., Ngadjui, B., Fomum, Z., Etoa, F-X. (2006). Evaluation of antidiarrhoeal activity of the fruit-rind of Picralima nitida (Apocynaceae). *African Journal of Traditional, Complementary, and Alternative Medicines*, 3(4):66-73

[10]. Otoo, LF., Koffuor, GA., Ansah, C., Mensah, KB., Benneh, C., Ben, IO. (2015). Assessment of an ethanolic seed extract of Picralima nitida ([Stapf] Th. and H. Durand) on reproductive hormones and its safety for use. *Journal of Intercultural Ethnopharmacology*, 4(4):293-301.

[11]. Bokota Twangaka M. Contribution à la caracterisation des principes actifs de Picralima nitida, plante utilisée par les pygmées (Mbute) pour combattre le paludisme à Mambasa en RD Congo | Centre d'Echange d'Informations de la RD Congo [Internet]. [consulted 2019 Jul 5]. Available from: http://cd.chm-cbd.net/implementation/centre-de-sureveillance-de-la-biodiversite-csb/chimie/chimie-organique/contribution-la-caracterisation-des-principes-actifs-de-picralima-nitida-plante

[12]. N'dri, F.K., Nene-Bi, S.A., Zahoui, O.S., Traore, F. (2015). Phytochemical and Toxicological Studies of an Extract of the Seeds of Picralima Nitida (Stapf) (Apocynaceae) and Its Pharmacological Effects on the Blood Pressure of Rabbit. *Journal of Biology and Life Science*, 6(1):116-128.

[13]. Lister, RG. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* (Berl), 92(2):180-5.

[14]. Chintawar, SD., Somani, RS., Kasture, VS., Kasture, SB. (2002). Nootropic activity of Albizzia lebbeck in mice. *Journal of Ethnopharmacology*, 81(3):299-305.

[15]. Une, HD., Sarveiya, VP., Pal, SC., Kasture, VS., Kasture, SB. (2001). Nootropic and anxiolytic activity of saponins of Albizzia lebbeck leaves. *Pharmacology Biochemistry and Behavior*, 69(3):439-44.

[16]. Pale, S., Kouemo, N., Taiwe, G., Kandeda, A., Okomolo, F., Njapdounke, J. et al. (2018). Antipyretic and Anxiolytic Properties of Aqueous Extract of Cymbopogon giganteus in Rats Model. *International Neuropsychiatric Disease Journal*, 11(1):1-10.

[17]. Seibenhener, M., Wooten, M. (2015). Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice. *Journal of Visualized Experiments*, (96), doi: 10.3791/52434.

[18]. Brown, G., Nemes, C. (2008). The exploratory behaviour of rats in the hole-board apparatus: Is head-dipping a valid measure of neophilia? *Behavioral Processes*, 78(3):442-8.

[19]. Kanyonga, P., Faouzi, M., Zellou, A., Essassi, M., Cherrah, Y. (2009). Évaluation de l'activité anxiolytique de la 4-phenyl-1,5-benzodiazepin-2-one. *International Journal of Biological and Chemical Sciences*, 3(4): 646-652

[20]. Menzies, J., Paterson, S., Duwiejua, M. Corbett, A. (1998). Opioid activity of alkaloids extracted from Picralima nitida (fam. Apocynaceae). *European Journal of Pharmacology*, 350(1):101-8.

[21]. Walf, A., Rhodes, M., Frye, C. (2004). Antidepressant effects of ER β-selective estrogen receptor modulators in the forced swim test. *Pharmacology Biochemistry and Behavior*, 78(3):523-9.

[22]. Antidépresseurs : Les points essentiels [Internet]. [consulted 2019 Jun 25]. Available from: https://pharmacomedicale.org/medicaments/par-specialites/item/antidepresseurs-les-points-essentiels

[23]. Levy, L., Degnan, A. (2013). GABA-based evaluation of neurologic conditions: MR spectroscopy. American Journal of Neuroradiology, 34(2):259-65.

[24]. Issa, T., Souleymane, F., Charlemagne, G., Eloi, P., Roger, N. (2016). Antiplasmodial and DPPH Radical Scavenging Effects in Extracts from Acacia macrostachya (Mimosaceae) DC. *World Journal of Pharmaceutical Research*, 5(8):219-233.

[25]. Pincemail, J., Degrune, F., Voussure, S., Malherbe, C., Paquot, N., Defraigne, J. (2007). Effet d'une Alimentation Riche en Fruits et Légumes sur les Taux Plasmatiques en Antioxydants et des Marqueurs des Dommages Oxydatifs. *Nutrion Clinique et Métabolisme*, 21(2):66-75.

[26]. Liao, B., Newmark, H., Zhou, R. (2002). Neuroprotective Effects of Ginseng Total Saponin and Ginsenosides Rb1 and Rg1 on Spinal Cord Neurons in Vitro. *Experimental Neurology*, 173(2):224-34.

[27]. pubmeddev, al DJ et. Tolerance liability of diazepam is dependent on the dose used for protracted treatment. - PubMed - NCBI [Internet]. [consulted 2019 Jul 24]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23238468