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CHEMICAL SCREENING AND ANTICONVULSANT ACTIVITY OF THE AQUEOUS EXTRACT OF THE BARKS OF TRUNK OF *PICRALIMA NITIDA* STAPF (APOCYNACEAE) IN ALBINOS MICE

Ngaïssona P.[1]*, Etou Ossibi A.W[1,3], Nkounkou Loumpangou C[1], Bassoueka D.J[3], Gouollaly Tsiba[1], Namkona F.A.[2], Koane J.N[2], Syssa-MagaléJ.L[2] and Ouamba J.M[1]

[1]Chemistry Unit of the Plant and Life, Faculty of Sciences and Technical, University Marien N'gouabi, B.P. 69, Brazzaville – Congo.

[2]Laboratory of Architecture, Analysis and Reactivity of Natural Substances, Faculty of Sciences, University of Bangui, B.P 908 Bangui.

[3]Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences, University Marien N'gouabi, B.P. 69, Brazzaville – Congo.

***Corresponding Author: Ngaïssona P.**

Chemistry Unit of the Plant and Life, Faculty of Sciences and Technical, University Marien N'gouabi, B.P. 69, Brazzaville – Congo.

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ABSTRACT

Picralima nitida (Stapf) Th. and H. Dur. (Apocynaceae) is a plant of the Central African Pharmacopoeia that the barks of trunk are widely used in traditional medicine for several diseases and convulsive seizures. The main objective of this study was to assess the anticonvulsant effects of the aqueous extract of the barks of trunk of this plant. As a preliminary, a chemical screening by reactions in tube and a study of acute toxicity in mice according to OECD, (2001) were carried out. The anticonvulsant activity of the aqueous extract in the doses of 250 and 500 mg/kg was estimated against the convulsions induced by strychnine (3 mg/kg, i.p). Preliminary phytochemical analysis showed the following major chemical families: alkaloids, gallic tannins, triterpenes and sterols, flavonoids, catechic tannins and saponins. This study also reveals that the aqueous extract of *Picralima nitida* Stapf is non-toxic orally in mice. The aqueous extract at a dose of 500 mg / kg body weight had a significant effect on the convulsion induced by strychnine. The results indicate the aqueous extract of the bark of the trunk of *Picralima nitida* Stapf appear to possess anticonvulsant properties similar to the reference molecule diazepam (Valium®).

KEYWORDS: *Picralima nitida* Stapf, secondary metabolites, acute toxicity, anticonvulsant, spontaneous locomotor.

INTRODUCTION

Traditional medicine nowadays remains the principal solution of a majority of Sub-Saharan populations in general and the Central African Republic in particular for the assumption of responsibility of much pathology like the diabetes, arterial hypertension, malaria, the epilepsy, the bacterial infections, etc. These populations use to this medicine because of the lack of access to the conventional drugs; of a real effectiveness of the medicinal herbs and their low costs (Lhuillier, 2007; Koné, 2009). The effects of these plants are due to their wealth of made up bioactive such as the flavonoids, alkaloids, triterpenes and sterols, tannins, saponins, etc. (Leven and *al*., 1979).

In the Central African Republic, the country with high malaria endemicity, febrile convulsions of malaria origin are estimated to 33.5% of cases (Nambei and *al*., 2013, Bobossi-Serengbe and *al*., 2015). The convulsions affect the children at low age and occur at the time of a rise in the body temperature (Pedspan, 2007). They can also be caused by fever outside the central nervous system (Alajlouni and *al*., 2000; Ling, 2001). Most modern drugs (anticonvulsant) prescribed to treat the convulsions have harmful side- effects. Some plants such as *Picralima nitida* Stapf (Apocynaceae) are used in the treatment of the convulsions seizures in Central Africa (Ake-Assi and *al*., 1978; Adjanohoun and *al*., 1979).

Picralima nitida Stapf., also called *Tabernaemontana nitida* Stapf., is very wide-spread in tropical and subtropical Africa. This plant is characterized by hard and fragile barks, opposite large sheets. Barks are used in traditional medicine against the malaria, the diabetes, the typhoid fever, the hypertension, the stomach pains, the convulsions, etc. (Burkill, 1985, Adjanohoun and *al*., 1996, Kouitcheu and *al*., 2008, Jiofack and *al*., 2009, Koffi and *al*., 2014, Akunne and *al*., 2015). The present study would like to make a contribution to the scientific valorization of the Central African pharmacopeia. Thus,

it was aimed to estimate experimentally the anticonvulsant effects bark of trunk of *Picralima nitida* Stapf in the mouse.

MATERIALS AND METHODS

Plant material

The bark of the trunk of *Picralima nitida* Stapf, were used. These barks were collected at Yamboro village at 18 ° 23'6 "East longitude and 4 ° 19'23 " South-East latitude in the region Ombella M'poko, 30 km south of Bangui (Central African Republic). The botanical identification of this plant was made at the Laboratory of Plant Biology of the Faculty of Sciences of the University of Bangui. The bark of trunk were dried out of sun and moisture then crushed. The powder obtained was preserved in glass bottles for the later analyses.

Animal material

Males and females Swiss Albino mice adult of ranging between 19 - 29 g were used. These mice, provided by the animalery of the Faculty of Sciences and Technology of Marien N'gouabi University (Congo), were acclimatized to the Laboratory of Biochemistry and Pharmacology of the Faculty of Health Sciences of the University of Marien N'gouabi (Congo) for 5 days before the experiments. The animals were maintained under standard conditions (12 hours of lighting, 12 hours of darkness) and temperature of 27 ± 2 ° C with free access to standard food and tap drinking water.

Preparation of the aqueous extract

Fifty (50) grams of powder trunk bark of *Picralima nitida* Stapf., were submitted to a decoction in 500 mL of water distilled during 30 minutes. After cooling and filtration, the filtrate obtained was evaporated to dryness under reduced pressure in a type Büchi R-200 rotary evaporator at 60 ° C. The dry concentrate obtained was weighed and stored in a bottle out of glass for biological tests.

Preliminary phytochemical screening

The phytochemical screening of the extracts of *Picralima nitida* Stapf was carried out by the classical method described by Bouquet, (1969), Jonville, (2011) and Lusakibanza, (2012). It is about a qualitative analysis based on the reactions of colorings and/or precipitations with the specific reagents.

Evaluation of acute toxicity

The acute toxicity of the aqueous extract of the barks of trunks of *Picralima nitida* Stapf was evaluated in the mice, according to the guide line n°423 of December 17th, 2001 of OECD (Organization for Economic Cooperation and Development) relating to the tests of the chemical substances and according to the protocol describes by Lorke, (1983) with a slight modification. Nine (9) mice, beforehand put on an empty stomach during 18 hours before the experiment, were divided into three (3) groups of three (3) mice each one and treated by orally way as follows: the first group received water distilled (0,5 ml/100g, of body weight). The second and third groups received the single doses of 2000 and 5000 mg/kg of body weight of the aqueous extract of the barks of trunk of the plant, respectively.

After the administration of the products (water and extract), the mice were observed for thirty (30) minutes, then at every hour during four (4) hours. These observations related to the ptosis, aggressiveness, mobility, vigilance, the vomiting, vocalization, the state of the saddles and the convulsions. The number of mice died in each batch was noted during 48 hours after the administration of the products.

Test of the convulsions induced by strychnine

Twenty-four (24) mice were divided into four (4) groups of six (6) mice each one were treated in the following way: The first group received distilled water (0.5 ml/100 g of body weight), the second group received the diazepam (molecule of reference) with 10 mg/kg of body weight. Finally, the third and fourth groups received the aqueous extract of *Picralima nitida* Stapf in the respective doses of 250 and 500 mg/kg of body weight. One hour after oral administration of the products, the mice of groups 2, 3 and 4, had received strychnine (3 mg/kg, i.p). The mice which had not convulsed or which after the convulsions had not died within 10 minute after the administration of strychnine were regarded as protected Ngo Bum and *al*., (2001); Yemitan and *al*., (2005). The percentage of protection was calculated according to the following formula:

$$Percentage\ \ protection = \frac{number\ of\ \ protection}{n} \times 100$$

$$with\ n = 6$$

Test of the spontaneous motor activity

This test was carried out according to the method described by Martin and *al*., (1990) with a slight modification (Abena and *al*., 2004). The mice were previously fasted during 18 hours before the experimentation with free access to drinking water. Then, twenty (20) mice divided into four (4) batches of five (5) mice each one were treated by oral way in the following way: group 1 witness received distilled water (0.5 ml/100 g of body weight), group 2 received Diazepam (Valium ®) at 10 mg/kg of body weight and groups 3 and 4 received the aqueous extract of *Picralima nitida* to the respective doses of 250 and 500 mg/kg of body weight.

One hour after the administration of the products, each mouse was placed in a cage with motor activity of dimensions: length: 43.3 cm; width: 27.6 cm and height: 15.2 cm having a floor squared (6.9 × 6.8 cm^2). Then, the number of rectangles crossed by each mouse was counted during five (5) minutes.

Analyze results

The results are expressed on average affected standard error on the average. The comparison of the averages between the treated and pilot batches was made by application of the test of Student, follow-up of the analysis of variance (ANOVA). The significance limit was at set $p < 0.05$.

RESULTS

Phytochemical screening of extracts of *Picralima nitida* Stapf

The results of the preliminary phytochemical tests carried out on the extracts of the barks of trunk of *Picralima nitida* Stapf are presented in table I. We note that the alkaloids, gallic tannins, the triterpenes and sterols are very abundant in the extracts of barks of trunk of *Picralima nitida* Stapf., compared to flavonoids, catechic tannins and saponins. We also note the total absence of the anthocyanins, quinones and reducing sugars.

Table I: Results of the phytochemical screening of the extracts of the bark of the trunk of *Picralima nitida* Stapf.

Chemical groups	*Picralima nitida* Bark of the trunk
Alkaloids	+++
Flavonoids	++
Quinones	-
Catechic tannins	++
Gallic Tannins	+++
Saponins	++
Terpenoids	+++
Anthocyanins	-
Reducing sugar	-

+++: Very abundant ++: presence +: trace – Negative

Acute Toxicity of the aqueous extract of *Picralima nitida* Stapf.

The oral administration of the aqueous extract of *Picralima nitida* Stapf (2000 and 5000 mg/kg) does not cause a change in the behavior or of the general state of the mice relative to the control. Moreover, no mortality was observed up to 48 hours as in the mice of the control. However to mice having received the extract at 5000 mg/kg, it was recorded the death of two (2) mice in the fourteenth day.

We followed the evolution of the weight in the mice submitted for testing, the days (J_0 to J_{14}) for the acute toxicity test. The results are grouped on the figure 1, representing the relative weight of the mice (%) function of the day.

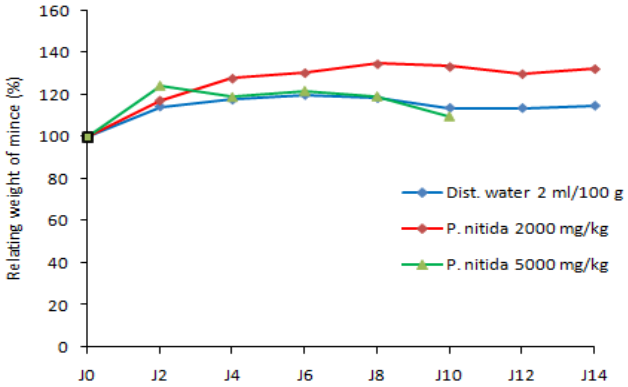

Figure 1: Effect of the aqueous of P. nitida on the weight evolution of the mince

Anticonvulsants effects of the aqueous extract of *Picralima nitida* Stapf.

Table II shows the effects of the aqueous extract of *Picralima nitida* Stapf on the convulsions induced by strychnine in the mouse. From this table, the aqueous extract of *Picralima nitida* Stapf at 500 mg/kg and the diazepam (10 mg/kg) increase the time of onset of the convulsions to 528.66 ± 71.33 ($p < 0,05$) and 532.16 ± 43.76 dryness ($p < 0.01$) compared with control 253 ± 50 dryness. The respective percentages of protection are 83.33 and 66.67 %. At the dose of 250 mg/kg, this extract cause a non-significant variation of the time of appearance of the convulsions with a null percentage of protection of the mice.

Table II: Effects of the aqueous extract of *Picralima nitida* on the convulsions induced by strychnine.

Treatment	Dose (mg/kg) Oral route	Time of onset of convulsion (sec) ± Sem	% of protections
Distilled water	0,5 ml/100 g	253 ± 50	0
Diazepam	10	532.16 ± 43.76 **	66.67
Picralima nitida	250	245.66 ± 19.55 NS	0
Picralima nitida	500	528.66 ± 71.33 *	83.33

The values are mean ± Sem for n = 6; * P <0.05; ** p <0.01 significant difference from control (distilled water) and NS: Not significant.

Effects of the aqueous extract of *Picralima nitida* on spontaneous motricity

The results of the effect of the aqueous extract of *Picralima nitida* Stapf on the spontaneous motor activity are presented on figure 2. It appears from this figure that, like the diazepam (at the dose 10 mg/kg), the aqueous extract of *Picralima nitida* Stapf at a dose of 500 mg/kg causes a significant reduction in the number of rectangles crossed by the mice relative to the controls. This number increased from 170 ± 5,43 in the control to 115.60 ±

10.37 ($p < 0.001$) and 143.60 ± 4.32 ($p < 0.001$) respectively in the mice treated with the diazepam (10 mg/kg) and the extract (500 mg/kg); respectively decreases of 32 and 15.53 %. This extract with 500 mg/kg thus decreases the spontaneous locomotor activity of the mice.

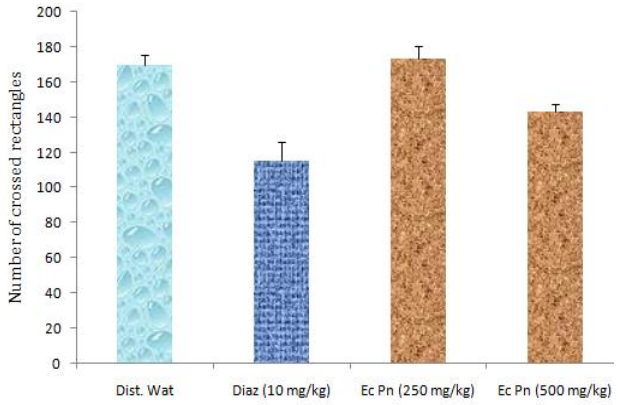

Figure 2: Effects of the aqueous extract of *Picralima nitida* Stapf (Pn) on the number of rectangles crossed by the mice. Diaz: diazepam; Dist. Wat: distilled water; Values are means ± ESM, with n = 5; *** p < 0,001 significant difference from control (distilled water); ns: difference not Significant compared to control (distilled water).

DISCUSSION

The results of the phytochemical screening show that the barks of trunk of *Picralima nitida* Stapf are rich in secondary metabolites as shown by an earlier phytochemical study (Ngaissona and *al*., 2015). The presence of these chemical families in the leaves and the barks of trunk of varieties of the species of Nigeria and Cameroun have also been shown. Former Works highlighted in seeds of the species of Ivory Coast alkaloids and the terpenoids Ilodigwe EE and *al*., (2012); Teugwa and *al.*, 2013; Kouassi and *al.*, (2015).

The absence of signs of toxicity observed suggests that the aqueous extract of the barks of trunk of *Picralima nitida* Stapf at doses of 2000 and 5000 mg/kg is well tolerated in the mouse. Up to the dose 5000 mg/kg, no mortality was observed. This result suggest that the lethal dose 50 (DL_{50}) of the aqueous extract of this plant would be higher than 5000mg/kg of body weight. It should be noted, that it was shown that the values of DL_{50} lower than 5000 mg/kg correspond to highly toxic substances and those higher than 5000mg/kg with the substances slightly toxic (Delongeas and *al*., 1983; Diezi, 1989). The aqueous extract of the barks of trunk of *Picralima nitida* would be thus slightly toxic. Work of Ilodigwe and *al*., (2012); Kouassi N'dri and *al*., (2015); Akunne and *al*., (2015) carried out on the ethanolic, methanolic and aqueous extracts of leaves, seeds and roots of *Picralima nitida* Stapf showed similar results. On the other hand, the study of the acute toxicity of the extracts methanolic and aqueous of the fruit pulp of *Picralima nitida* Stapf at a dose 5000 mg/kg revealed signs of toxicity and death in white albino mice after 10 hours of administration of these extracts (Namdi and al. , 2015).

The aqueous extract of *Picralima nitida* Stapf, significantly decreases the spontaneous motor activity as does the reference molecule, the diazepam. Concerning the anticonvulsant activity, the results obtained show that this extract at 500 mg/kg, as the diazepam, increases the time of appearance of the convulsions induced by the strychnine, with a significant percentage of protection. The aqueous extract of the barks of trunk of *Picralima nitida* Stapf seems to possess properties anticonvulsant as diazepam, a known anticonvulsant. The works done by Biggio and *al*., (1992); Adeyemi and *al*., (2010) showed that strychnine is one powerful convulsant of the spinal cord, which blocks receivers of the glycine in a selective way to induce an exciting answer in the central nervous system and increases the spinal reflexes (Row and *al*., 1998). The anticonvulsant activity of this extract would be due probably to the presence in this plant of the secondary metabolites such as alkaloids and the terpenoids (Gareri and *al*., 2004; Bhutada and *al*., 2010; Kumar and *al*., 2012).

Note that according to Kumar and *al*., (2012) various classes of molecules such as alkaloids, lipids, terpenes, triterpenoids, flavonoids and coumarins would have anticonvulsant properties.

CONCLUSION

The barks of trunk of *Picralima nitida* Stapf., are rich in secondary metabolites. The aqueous extract of these barks is well tolerated in the mouse up to 5000 mg/kg. In addition, this extract with at a dose of 500 mg/kg of body weight protects from the convulsions induced by strychnine and decreases the spontaneous motor activity of the mice. These results show that this extract possesses properties anticonvulsants and sedative which could partly justify its use in traditional medicine against the epilepsies. However, pharmacological tests with other convulsants agents deserve others realized.

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