Evaluation of the Antioxidant Capacity and Studies of the Anticonvulsant Properties of *Hymenocardia acida*

Abstract

The work focuses on evaluating the antioxidant capacity and studying the anticonvulsant properties of the bark of *Hymencardia acida* (Euphorbiaceae), which is a tropical African plant traditionally used in the Centre Region of Cameroon in the treatment of many diseases such as migraines, diarrhoea, wounds, stomach ache and influenza. The various antioxidant tests carried out have highlighted the high antioxidant content of the bark of these plants. The results of the anticonvulsant activity showed that: The decoction of *Hymencardia acida* antagonized the convulsions induced by 100% pentylenetetrazole at doses of 22.5, 45 and 90 mg/kg. It also protected mice from convulsions induced by pilocarpine, 100% at doses 45 and 90 mg/kg and in the 4-aminopyridine convulsion induction test, it protected mice 100% at doses 45 and 90 mg/kg with a significant difference of $(P \leq 0.001)$ compared to the negative control. This study justify the use of **H acida** in traditional medicine category 1, for the treatment of epilepsy in the community of the Centre Region of Cameroon.

Keywords: epilepsy, convulsion, anticonvulsant, decoction, *Hymenocardiaacida*

Introduction

Hymnocardiaacida is a shrub or small dioecious tree, deciduous in nature, reaching (6-10) m in height, often diffuse; shaft up to 30 cm in diameter, often stunted or twisted; smooth, light brown or grey bark, peeling off to reveal a powdery, reddish to orange inner bark; upper branches spreading out, lower branches drooping; young shoots with short hairs. Leaves alternate, simple and entire; stipules 1-3 mm long, linear, rapidly dropping; petiole 0.5-1.5 cm long, short-haired; blade elliptic ovate to oblong-oblanceolate, 2.5-9.5 cm × 1.5-5 cm, base rounded, apex rounded to obtuse, short-hairy to almost glabrous above, densely soft-hairy below, or almost glabrous except for the main veins, punctuated with rare to evenly distributed yellowish glands (Orwa and al., 2010).

The bark of *H. acida* is used to treat diarrhoea and dysentery and shows good results. In Zambia, the bark is used in concoction as a remedy to treat illnesses so the origins and causes are unknown. In East Africa, it is used in wound treatment. Root bark extracts have shown

cytotoxicity against 60 human cell lines from the National Cancer Institute (NCI). *H. acida* extracts have shown activity against **klebsiella pneumonia**. *H. acida* is also used in Cameroon especially in the Mbam and Inoubou region by traditional practitioners to treat several ailments including colds, headaches and epilepsy (traditionalist, 2019). According to our investigations, there is also knowledge of the use and effectiveness of this plant in the treatment of rebellious coughs and tuberculosis in Central Africa, just as it is known in Ivory Coast for its great therapeutic virtues against malaria. The objective of this study was to evaluate the Antioxidant Capacity and Studies of the Anticonvulsant Properties of *Hymenocardia acida*

Materials and Methods

Animal material

For the performance of our pharmacological tests, white *Mus musculus* Swiss mice, approximately 3 months old with a body mass of 20 g to 25 g were used in the various pharmacological tests and for the toxicity test male and female rats of the **Wistarstrain** with an average weight of 100 g. They came from the toxicology laboratory of the Biochemistry Department of the Faculty of Sciences of the University of Yaoundé 1 where they were raised in plexiglass cages and fed with the granules whose composition was recommended by the said laboratory.

Plant material

The bark of *H. acida* was harvested in the region of Central Cameroon more precisely in the locality of Mbola in the Mbam &Inoubou, Division of Bokito District, in May 2019. *H. acida* was identified in reference to the botanical sample of ***Hymenocardiaacida Tul* (euphorbiceae)**, under the registration number 46856 at the National Herbarium of Cameroon (HNC) in Yaounde.

Chemical material

The chemical material consists of central nervous system depressants such as phenobarbital (Rivotril®, Neuilly-sur-Seine, France) and diazepam (Valium®, Fontenay-sous-Bois, France), and central nervous system stimulants such as pentylenetetrazole (MP

Biomedicals, IIIKrich, France), pilocarpine (Sigma Chemical, Saint louis, USA) and 4-aminopyridine (Sigma Chemical, Saint louis, USA).

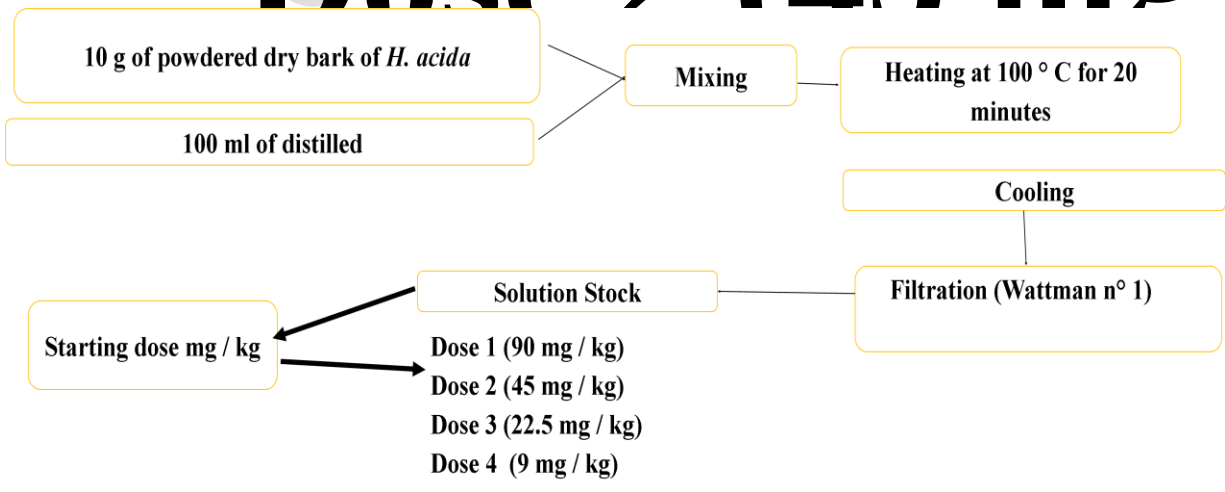
Statistical Analysis

The parameters measured in our experiments were: the percentage of protection against convulsions induced by the convulsive substances, the duration of the convulsions, the time and the body mass of the mice. The exact Fisher's test was used to compare the percentages of protection of the negative control mice with those of the test batches. An analysis of variance followed by the Tukey multiple comparison test was performed to compare the other parameters using XL software. Stat 2011 software. From $P < 0.05$ the values are considered to be different. (Konaté et al., 2011)

Preparation of *H. acida*bark decoction

H. acida barks are washed beforehand and dried in the shade for 14 days. The dry bark was crushed and then sieved with a 0.5 mm mesh sieve to obtain a fine powder. 10 g of this powder was introduced into a beaker containing 75 ml of distilled water, the whole was boiled for 20 minutes on a hot plate (brand IKA-COMBIMAG RCT, 220V, 50Hz, 630W) set at a temperature of 100°C. After cooling and filtering the mixture using Watman filter paper number 1, the filtrate (brown colour) whose volume has been measured (20 ml) is evaporated in the oven at a temperature of 50°C for 24 hours. 5.4 g of dry extract of *H. acida*was obtained, giving a yield of 5.4%.

For each subsequent dose, a mass of 10 g of *H. acida* bark powder was placed in a beaker containing 100 ml of distilled water. The decoction and filtration were carried out as before. The filtrate obtained constitutes the stock solution with an administration dose of 90 mg/kg, since the administration volume is 10 ml/kg. From this stock solution, successive dilutions with distilled water were made at 1/2, 1/4 and 1/10 to obtain the



doses 45, 22.5 and 90 mg/kg.

Chart 1. Preparation of ***H. acida*bark** decoction

Assessment of antioxidant potential

Evaluation of the antioxidant potential by the ABTS method

A first estimate of CA (antioxidant capacity) was made using the ABTS radical as follows: 0.07 g of potassium persulphate was added - as an oxidising agent - to 38.4 mg of azinobis (3-ethylbenzothiazoline-6-sulphonate) ammonium salt in 10 mL of distilled water to form the aqueous solution of ABTS. The mixture was left in the dark for 16 hours. For the CA measurement, 2 mL of the ABTS solution was diluted in 60 mL of distilled water to give initial absorbance values of 0.700 ± 0.010 at 734 nm. Then 200 µl of the sample was added in a plastic cuvette to 2 ml of the ABTS solution and the absorbance was read off after 15 min. A standard calibration curve was constructed with different concentrations. The CA was expressed in Trolox equivalent mM Trolox (Trolox equivalent antioxidant capacity, TEAC) (Rashidinejad et al., 2010).

Assessment of antioxidant potential by the DPPH method

The measurement of HPPD radical trapping was carried out according to the methodology described by Mollavali et al (2016). A 0.5 mM solution of DPPH was prepared in methanol on a daily basis. The reaction mixture consisted of 0.5 mL of sample, 3 mL of absolute ethanol and 2 mL of the DPPH solution. The blank was prepared by mixing 3.3 mL methanol and 0.5 mL sample. While 1 mg/ml was used as a control. The absorbance was read at 517 nm after 30 min of dark reaction. CA was calculated as a percentage according to (Djacbou et al., 2013) :

AA% = 100 - ((Abs sample - Abs white) x 100) / (Abs control) .

A calibration curve has been drawn (different concentrations according to their AA%). The final results were expressed in µM equivalents.

Assessment of antioxidant potential by the FRAP method

The FRAP test was done according to Sawant et al (2013). To prepare the FRAP solution: 10 mM TPTZ (2,4,6-Tris (2-pyridyl)-s-triazine) and 20 mM ferric chloride were diluted in 300 mM sodium acetate buffer adjusted to pH 3.6. 2ml of the FRAP solution was placed in a vial and 75 µl of the sample was added. The absorbance of the mixture was measured at 593 nm after 12 minutes of reaction. Gallic acid was used as the standard at different concentrations. The CA was expressed in mM.(Sawsan et al., 2018)

Evaluation of the antioxidant potential of phenolic compounds

The Folin-Ciocalteu reagent method has been carried out according to Mishra and his collaborators (2013). 20ml of distilled water was added to 100 µl of sample extract and 1 mL of the commercial Folin reagent. The mixture was stirred and allowed to react for 4 minutes, to which 400 µl of a **NaC03** solution (2 g/L) was added. The mixture was stirred and allowed to react for 100 minutes in the dark. Then the sample was centrifuged at 7000 rpm for 7 minutes at 4°c. Absorbance was measured at 760 nm *(Dubeau et al., 2010).* A standard calibration curve was constructed with different concentrations of gallic acid. The CA was expressed in mg of gallic acid equivalent/L.

Anticonvulsant tests

During our various anticonvulsant tests, the decoction was administered by gavage (*per os*) and the reference chemicals were administered intraperitoneally at a dose of 10 mg/kg.

Convulsion induction test with pentylene tetrazole (PTZ)

The pentylenetetrazole seizure induction test consists of inducing tonic-clonic convulsions in mice within 10 min by intraperitoneal administration of PTZ (selective GABA receptor antagonist) (70 mg/kg). The PTZ was injected one hour after treatment of the mice (seven mice per batch) with distilled water (10 mg/kg; *p.o.*) for the mice of the negative control batch, diazepam (0.1 mg/kg; i.p.) for the mice of the positive control batch and *H. **acida*****decoction*** *(*9; 22.5; 45; 90 mg/kg; *p.o.) for the mice of the* test batches. An episode of

spasm lasting at least 5 s was considered to be the onset of tonic-clonic convulsions. The mouse was said to be protected when it did not convulse within 10 min (*Ojong et al., 2018).*

The percentage of protection in each group was calculated according to the following formula: **percentage of protection = [(number of protected mice in a batch/total number of mice in the batch) × 100**].

Pilocarpine Seizure Induction Test

Intraperitoneal injection of pilocarpine in mice causes an evolving limbic crisis which will culminate in a generalised tonic-clonic convulsion. The mice were divided into six batches of 8 animals each. The first batch was treated with distilled water (10 ml/kg; *p.o.*), the four test batches with ***H. acida*decoction** (9; 22.5; 45; 90 mg/kg; *p.o.*) and the last batch with diazepam (5 mg/kg; i.p.). Thirty minutes after this treatment, atropine (1 mg/kg; i.p.) was administered to prevent the muscarinic peripheral effects of pilocarpine (diarrhoea, pilo-erection, mastication, tearing, trembling, spasms etc.). Thirty minutes later, pilocarpine (300 mg/kg; i.p.) was administered, and each mouse was observed for 1 h in a transparent Plexiglas cage (30 × 30 × 20 cm).

The evolution of the seizure was measured using a 5-stage scale: stage 1 (hyperactivity, shaking and/or convulsions and vibrational clones); stage 2 (head shaking, nodding and myoclonic convulsions); stage 3 (unilateral forelimb convulsions); stage 4 (recovery with bilateral forelimb convulsions); stage 5 (generalised tonic-clonic convulsions with loss of the righting reflex). Mice that did not convulse or die were said to be protected against myoclonic, clonic and tonic clonic convulsions. For each batch of mice, the percentage of protection was determined. In addition, the number of deaths was determined (for surviving batches), 60 min and 24 h later. The percentage of protection against death in each case was thus calculated. (Ngo Bum et al., 2011).

Convulsion induction test with 4-aminopyridine (4-AP)

Administration of 4-AP in mice causes clonic convulsions, followed a few seconds later by tonic convulsions. To evaluate the effect of the decoction on the convulsions induced by 4-AP. At first glance, the mice were formed in batches as before. However, the mice in the positive

control batch were given phenobarbital (30 mg/kg; i.p.). One hour later, 4-AP (voltage-dependent potassium channel antagonist) was administered intraperitoneally at a dose of 15 mg/kg to induce tonic-clonic convulsions. The tonic convulsions were characterised by a wild run, followed by tonic extension of the limbs for at least 5 s. The clonic convulsions were characterised by facial mycology, foreleg clones and hind limb clones for at least 5 sec. Immediately, the mice were observed individually in a Plexiglas cage for 30 min, for possible signs of changes in motor behaviour. Mice that did not convulse or die within 30 min were said to be protected *(Ojong et al., 2018).* The percentage of protection in each group was noted.

Results

Antioxidant activity of Hymenocardiaacida extract.

The analysis of the results showed that the plant had a much greater antioxidant activity than gallic acid as shown in table 1. This could be explained by the difference in the concentrations of the doses used to make the curves, so it would be wise to use the same concentrations in order to have comparable IC50s.

Table 1: Summary of the antioxidant activity of hymenocardiaacidaextract

Settings	DPPH	FRAP	ABTS
Gallicacid (µM)	1,031	16310	1,026
***Hymenocardiaacida* (µM)**	0,153	3,71e0^{,06}	0,026

Testing of phenolic compound

Table 2: Testing of phenolic compounds

Phenolic compound test					
conc mg/ml	1000	500	250	125	62,5
D.O. control	1,314	0,758	0,427	0,27	0,226

D.O plant	0,885	0,55	0,345	0,23	0,209

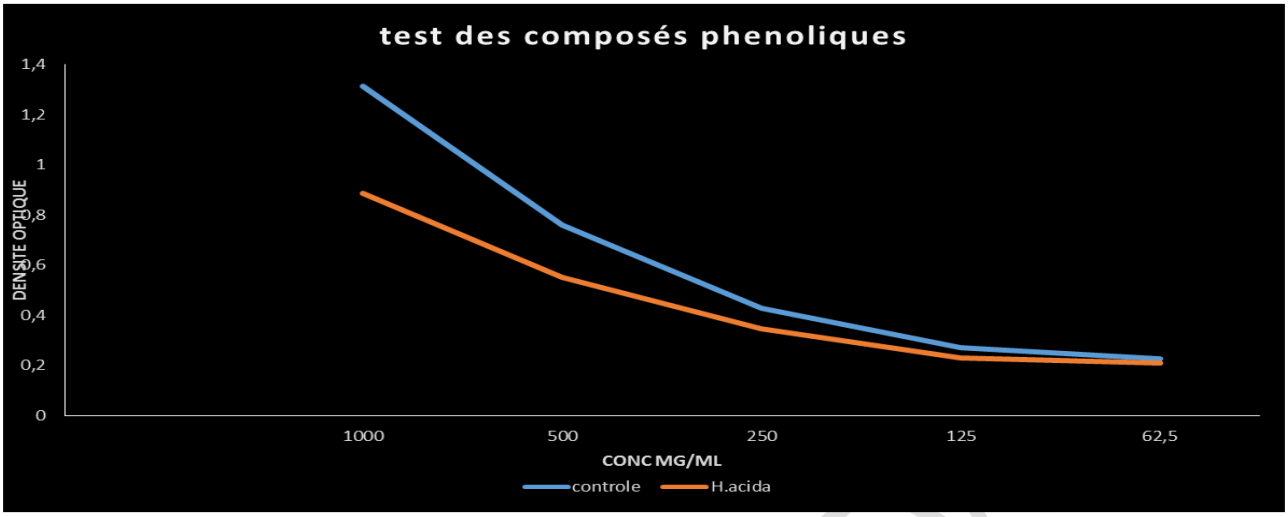
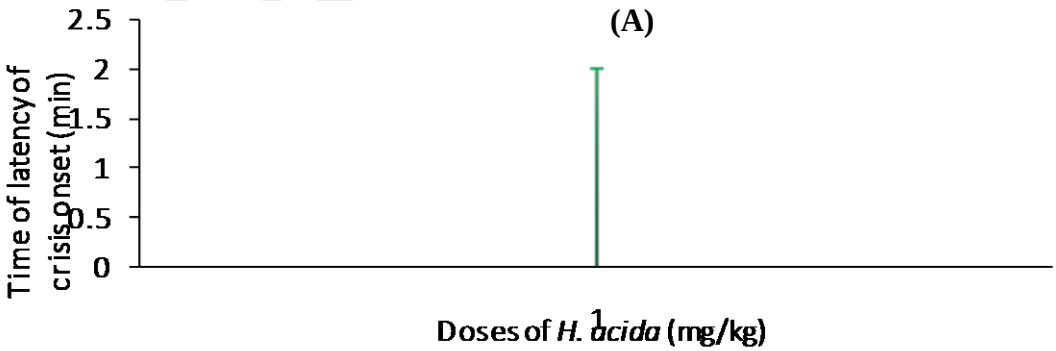


Figure 1: Antioxidant capacity of phenolic compounds

Anticonvulsant effect of *H. acida*

Effect of *H. acida*on Pentylene Tetrazole (PTZ)-induced clonic convulsions

Administration of PTZ (70 mg/kg) to mice previously treated with distilled water caused clonic convulsions in all mice in the batch treated with distilled water in less than 5 min. Pre-treatment of the animals with *H. acida*bark decoction induced significant protection *(p < 0.001) of* the mice against PTZ-induced convulsions of 100% at doses 9; 22.5; and 45 mg/kg similar to that observed in the batch that received the reference antiepileptic Diazepam (Figure 5).



(B)

Figure 2: Effects of H. *acida*on PTZ-induced clonic convulsions. (A): seizure onset latency time, (B): percentage of protection Each bar represents the mean ± ESM, n = 7. *p < 0.05; **p < 0.01; ***p < 0.001; significant difference from negative control. $_{H2O}$: Distilled water; DZP: Diazepam

Effects of *H. acida* on Pilocarpine-induced convulsions (PC)

Administration of CP at a dose of 300 mg/kg causes to nicoclonic (generalised) convulsions in all animals in the negative control group treated with distilled water. Decoction of *H. acida* bark at 45 and 90 mg/kg completely protected (p <0.001) 100% of the mice in these batches from CP-induced generalised convulsions. Whereas those in the batch receiving DZP had 60% protection (Figure 3A). Similarly, Figure 3B reports the number of animals protected against CP-induced convulsions and death. This number is three times higher in the mice receiving *H. acida* decoction at 45 and 90 mg/kg and one time higher in the mice receiving diazepam compared to the batch of animals treated with distilled water. However, after 24 h, the percentage of protection was higher in the test batches which received the *H. acida* decoction at 45 and 90 mg/kg respectively compared to the batch treated with distilled water (0%) or DZP (40%).

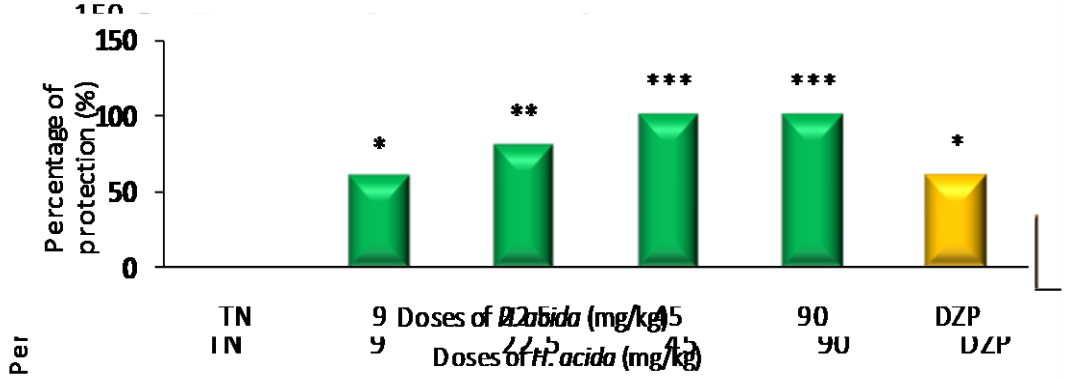


Figure 3: **Effects of *H. acida* on generalised convulsions induced by CP (A):** Percentage of protection of mice against convulsions; (B): Percentage of protection after one hour and after 24 hours. Each bar represents the **mean ± ESM,** n = 8. *p < 0.05; **p < 0.01; ***p < 0.001; significant difference from negative control. $_{H2O}$: distilled water; DZP: diazepam (0.3 mg/kg).

Effects of *H. acida* on the latency time of onset of CP-induced generalised convulsions

Figure 4; shows the latency time for the onset of convulsions. The latency time increased significantly *(p < 0.001) and* is three times longer in the test batches the positive control (DZP) than the control batch treated with distilled water. It went from 0 to 10 min respectively for doses 9; 22.5 and 45 mg/kg of the ***H. acida*decoction** and to 06 min for the 90 mg/kg dose.

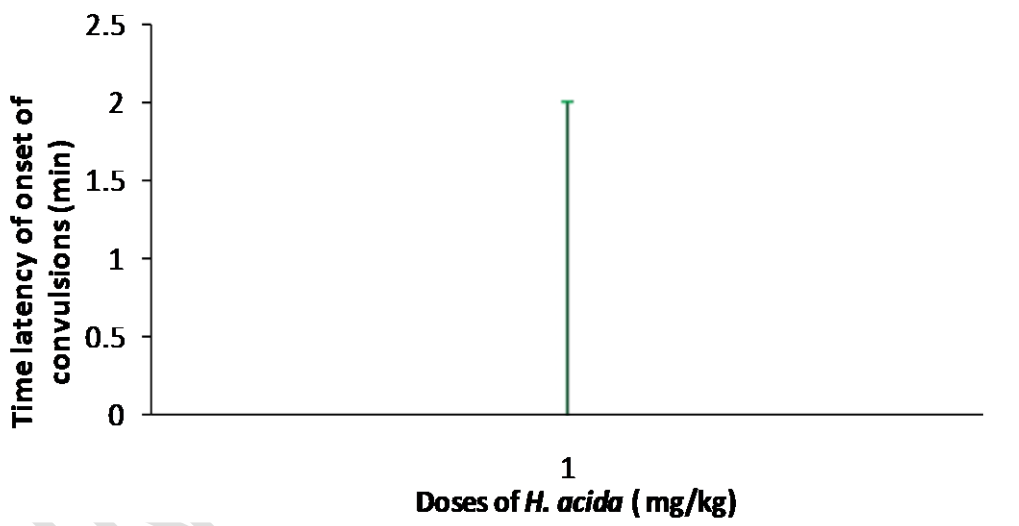


Figure 4: Effects of *H. acida*on the latency time of onset of MCS-induced generalised convulsions. Each bar represents the mean ± ESM, n = 8. *p < 0.05; **p ≤ 0.01; ***p ≤ 0.001; significant difference from negative control. $_{H2O}$: distilled water; DZP: diazepam (0.3 mg/kg) .

Effects of *H. acida*on tonic/clonic convulsions induced by 4 Aminopyridine (4-AP)

4-AP (12 mg/kg) induced tonic/clonic convulsions and death in all the animals in the negative control batch that received distilled water. Pretreatment with *H. acida*bark decoction

significantly *(P<0.001)* protected the animals from 4-AP-induced tonic/clonic convulsions. 4-AP led to an increase in the latency time of onset of convulsions (Figure 5). The increase in seizure onset latency time increased from 0 min for the control receiving distilled water for clonic and tonic convulsions respectively to three times the values of the positive test and control batches for clonic convulsions and similarly for tonic convulsion latency times.

Figure 5: Effects of *H. acida*on tonic/clonic seizures induced by 4-AP.

Effects of *H. acida* on the latency time of onset of tonic/clonic convulsions and on the duration of 4-AP-induced seizures

Figure 6 shows the effect of decoction on the duration of clonic crises. From 40 min for the batch treated with distilled water, the duration of the attack was significantly reduced *(P < 0.001) to* values of 3.75 ± 8.85 for doses 45 and 9 mg/kg and for the reference batch, values three times lower than those of the control: the effect of the plant would therefore be even better than that of phenobarbital.

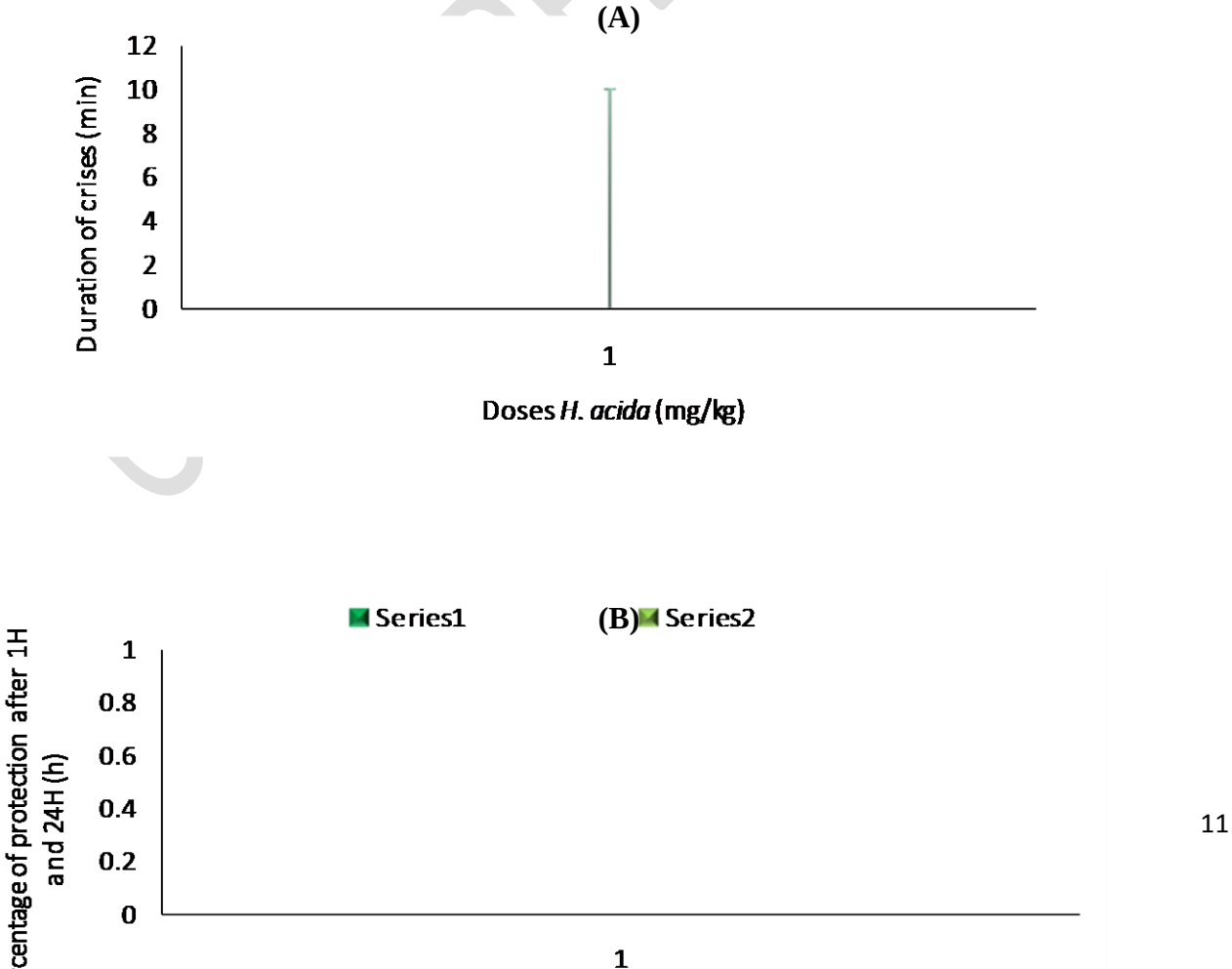


Figure 6: Effects of *H. acida*on the latency time of appearance of tonic/clonic convulsions induced by 4-AP. (A): duration of seizures, (B) percentage of protection. Each bar represents the mean ± ESM, n = 6. **p < 0.01; ***p < 0.001; significant difference from negative control. $_{H2O}$: distilled water; PB: phenobarbital (12 mg/kg).

Discussion

When epilepsy induced by chemical substances (PTZ, PC, 4-AP) occurs, a cascade of reactions with neurotoxicity occurs, leading to the death of several neurons and the release of free radicals. Antioxidant tests are thus carried out to determine the antioxidant potential of *H. acida*. In the ABTS test, *H. acida* showed an ability to turn the colour of the initially prepared ABTS radical to its original colourless neutral state, which shows that this plant possesses antioxidants that reacted with the cationic ABTS radical (Aliyu et al.., 2010) and the value of the concentration of *H.acida* obtained for ABTS is 0.026 µM which corroborates the results obtained by Sofidiya et al, (2014) who had obtained a 0.02 µM just as the value obtained in the work of (Olukemi et al., 2009) was 0.023. Similarly, the DPPH test showed the ability of *H. acida to* reduce the DPPH chemical radical by hydrogen transfer.

This shows that the extract is rich in antioxidants (Mustapha et al., 2016) and the DPPH value obtained is 0.153, which confirms the value found by Ngbolua et al. (2013) who found 0.155. In tests with FRAP and phenolic compounds, with FRAP, the extract was able to reduce ferric ions to ferrous ions (Hosseini et al., 2013) furthermore the FRAP value is 3.71e0.^{06} µM which is almost in line with the value found by Oluwakayode et al (2015) which is 3.19 $^{e0.08}$ µM and with the phenolic compounds test, the extract reduced the yellow coloured reagent to a mixture of tungsten blue oxide and molybdenum which is evidence of the presence of polyphenols (Eddine et al., 2018). *Moreover,* the value obtained for the phenolic compounds is 0.055 µM which almost confirms the work of (Mustapha et al., 2016) who found a value of 0.062 µM.

As far as anticonvulsant tests are concerned, the phytochemical screening carried out on the extract revealed the presence of alkaloids, quinones, glycosides, anthocyanins, saponins, tannins, flavonoids, phthobanins etc. (Shimizu et al., 2014) among which flavonoids and saponins because flavonoids act by blocking monoamine oxidase and Catechol-O-Methyl Transferase more particularly MAO-A; like saponins, they also act on the serotoninergic system, increasing the density of 5 HTI A and 5 HT2 A receptors, without modifying their affinity. It has

been shown that saponins have a strong affinity for GABAergic receptors, and that flavonoids have a strong affinity for benzodiazepine receptors. Because reduced the expression of cytokines, which are involved in certain forms of depression, they are known to act in the nervous system and as anticonvulsant active ingredients because thanks to their OH group they can trap free radicals present in the CNS. Flavonoids can inhibit the release of pro-inflammatory mediators (histamine, arachidonic acid derivatives, lysosomal enzymes, basophils, mast cells and neutrophils). Some of these appear to modify lymphocyte function and consequently the immunological response. It is thought that the anti-inflammatory effect is related to different mechanisms of action depending on the chemical structure, as the hydroxyl group binds to the gabergic receptors blocking the release of free radicals (Ojong et al., 2016).

We used 03 inducers of generalised tonic-clonic convulsions. PTZ is a GABA receptor antagonist convulsive chemical. When administered it binds to the **Gaba** receptor sites that were to be occupied by the Gaba reducing the membrane permeability of chloride ions. The ability of the plant to prevent or reduce the frequency of PTZ-induced convulsions shows that ***H. acida*acts** on the CNS via the **Gaba** receptor with anticonvulsant action (Ngo bum et al., 2008). The same antagonistic effect is observed in the CP-induced seizure disorder test, which is a substance that binds to muscarinic receptors in the CNS, reducing tonic-clonic seizures. The anticonvulsant activity carried out by *H. acida* and its ability to reduce PK-induced seizures shows that it acts on the muscarinic glutamate receptors in the CNS and the hypo camber.

Similarly, the 4-AP-induced convulsion test has shown its ability to reduce the tonicoclonic seizures induced by 4-AP and to protect animals from death caused by this substance. 4-AP is an agonist that binds to acetylcholinergic receptors causing the opening of calcium channels. The ability of *H. acida*to reduce seizures and protect animals shows that *H. acida*acts on acetilcholinergic receptors in the CNS. All these actions suggest the antioxidant potential of *H. acida* and its action in the CNS on Gaba, muscarinic, and voltage-dependent potassium channels.

Conclusion

The present work on ***H. acida*decoction** was aimed at determining the antioxidant capacity and evaluating the anticonvulsant effects of *H. acida.* This shows that :

H. acida acts on the CNS via **gaba,** muscarinic and voltage-dependent potassium channels and has an anticonvulsant action. This would explain its use in traditional medicine for the treatment of epilepsy.This plant had a strong antioxidant capacity. At the end of this work, we have created a product derived from our plant that requires some improvements before its final use.

Abbreviations

DZP: Diazepam; PC: Pilocarpine; 4-AP: 4-Aminopyridine; H. acida: *Hymenocardiaacida*; C. A: Antioxidant capacity;

References

1. **Aliyu A. B., Ibrahim H., Musa A. M., Ibrahim M. A., Oyewale A. O., Amupitan J. O., 2010.** In vitro evaluation of antioxidant activity of *Anisopusmannii*N.E. Br. African Journal of Biotechnology 9(16) 2437-2441.
1. **Alvarez Vincent, beucler marc, borgeshelder, Tapparel Ludovic, 2004.** community immersion.
2. **Djacbou d., Penlap v., 2014.** Comparison of in vitro antioxidant properties of extract from three plants used in medical purpose in Cameroun: acalypha racemosa, garcinia lucida and hymenocardialyrata. Vol 4, supplement 2, 625 - 632.
3. **Dongmo L., Druet-Cabanac M., Moyou S.R., Zebaze D. R. M., Njamnshi A. K., Sini V., Mapoure N., Echouffo T. J. B., Djeumen W. C., Ndumbe P. M., 2004.** Cysticercosis and epilepsy: a case-control study in Mbam Valley, Cameroon. Bull Société de pathologie exotique, 97 (2). 105-108.
4. **Eddine S., 2018.** Phytotherapy and medicinal plants Wishes and priorities for SEP2D of the research teams, thesis 15-15. University of the Mentouri Constantine Brothers. Faculty of Natural and Life Sciences, Algeria.

5. **Hosseini., Harandizadeh., Niazamand., Soukhtanloo., Mahmoudabady. , 2013**, antioxidant effect of *achillea wilhelmsii*extract on pentylenetetrazole (seizure model) - induced oxidative brain damage in wistar rats. *Indian J PhysiolPharmacol 2013; 57(4): 418-424*
6. **Konaté A., Sawadogo WR, Dubruc., Caillard O., Ouedraogo M., Guissou IP., 2012**. Phytochemical and anticonvulsant properties of Annona senegalensis pers.(Annonnacea), Plant used in Burkina Folk Medicine to treat epilepsie and convulsions. British journal of pharmacology and toxicology3(5), 245-250.
7. **Meyer AC., Sokhi D., Ngugi A., Solomon T., Fèvre EM.,** global disparities in the epilepsie treatment gap: a systematic review. Bull world Health Organ,2010. 88:260-266.
8. **Mustapha M., Haruna A., Musa A., Aliyu M., 2018.** Evaluation of Anticonvulsant Activity of Methanol Leaf Extract ofHymenocardiaacida,Tul(Euphorbiaceae) in laboratory animals. *Journal of Pharmaceutical and Allied Sciences 14 (5) (2017) 2675 - 2683.*
9. **Mustafa A., Ali A., 2008.** Substance in broad beans (*Vicia faba*) is protective against experimentally induced mice convulsions in mice, Epilepsy Behav, 12 25-9.
10. **Mustapha M, Toumi B. Koudache F., 2016**. Optimisation of the quantification of polyphenols and their antioxidant activities in relation to altitude: case of *Daphne gnidium.*
11. **Ngbolua R S., Mudogo V., Takoy L.1, Rakotoarimana H.2*, Mpiana,** Evaluation of the antiseptic and anti-malarial activity of some plant taxa in the Democratic Republic of Congo and Madagascar
12. **Ngo Bum., Pelanken M., Njikam N., Talla E., Taiwe G., Nkantchoua C., Ngoupaye G.,** The Decoction of Leaves of *Phyllanthus discoideus*Possesses Anticonvulsivant and sedative Properties in Mice, 2009, *International Journal of Pharmacology, 5: 168-172*.
13. **Ngo Bum E., Taiwe S., Moto O., Ngoupaye T., Vougat N., Sakoue D., Gwa C., Ayissi R., Dong C., Rakotonirina A., Rakotonirina V., 2011.** Antiepileptic Medicinal Plants used in Traditional Medicine to Treat Epilepsy, has been accepted for publication in the book "Epilepsy/Book 3", ISBN 979-953-307-038-3
14. **Okpo SO., Ayinde BA., Nuka PA., Sode OS.,** Evaluation of anti-Inflammatory Activity of Methanol Sterm Bark Extract of Hymenocardiaacidatul(Euphorbiaceae). *Nigerian journal of pharmaceutical and applied sciences Research* 5 (1), 80-86.

15. **Olukemi O., Afolayan J., Familoni O., 2009.** Phenolic content, antioxydant and antibacterial activities of hymenocardiaacida, natural product Research 29(2), 268-177.
16. **Oluwakayode o.** ,**Obi g., Ogwuche c., Elemike E., Oderinlo Y., 2015.** Phytochemical screening, antioxidant and antimicrobial activities of *Senna ocicidentialis*(L.) leaves extract. Clinical phytoscience (1) 1-6.
17. **WHO, 2018;** Key benchmarks on epilepsy.
18. **Lucie Josiane Ojong, Jean Pierre Abdou. Nadège Gisele Claudine Nkantchoua2 , Roger SomoMoyou, Elisabeth Bum Ngo. ,** Anti convulsant and *in vitro* antioxidantactivitiesofMomordicacissoides L. (Cucurbitaceae)
19. **Ojong L., Ayissi M., Pahaye D., 2018.** Anxiolytic and antiepileptic properties of the aqueous extract of *Cissus quadrangularis* (vitaceae) in mice pilocarpine model of epilepsy.
20. **Orwa, 2009**. Hymenocardia. *Agroforestry Database 4.0. 2 - 5.*
21. **Rashinejada.** ,**Birch J., Everett D., 2017,** Addition of milk to trans infusins/ Helpful or harmful? Evidence from in vitro and in vivo studies on antioxidant properties. critical reviews in food science and nutrition 57(15),3188-3196.
22. **Sawsan A., Abuhamdah., Amjad N., Fadwa T., Suleiman A., Ennaceur A., Chazot P., 2018.** Anti-convulsant Effects of *Bongardiachrysogonum L. Tuber* in the Pentylenetetrazole-induced Seizure Model**,** *International Journal of Pharmacology, Volume 14 (1): 127-135*.
23. **Shimizu k.** ,**Akinobu T., Akira I., 2014**. Biological activiyies and phytochemical profiles of extracts from different part of bamboo *(Phyllostachys pubescens).* Molecular 2014, 19 (6), 8238-8260.

Sofidiya O., Essien I., Aigbe F., Chidebelu E., 2014. Anyinociceptive and anti-inflammatory activities of ethanolic extract of *Alafia barteri*, *RevistaBrasilrira*, de farmacognosia 24(3),3