IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 17, Issue 6 Ser. III (Nov. – Dec. 2022), PP 43-55
www.Iosrjournals.Org

Phytochemical study, antioxidant and anticonvulsant activities of aqueous extract of leaves of *Opilia celtidifolia* (guill. Et perr.) Endl. Ex walp. Opiliaceae, from Benin.

DASSOU E. Morel[1a], GLINMA Bienvenu[1a,b*], ASSOGBA M. Fidèle[1a], AKAKPO B. Huguette[1a], TOKLO Placide M.[1a], AHOTON Djidénou[1a], KPOVIESSI D.S. Salomé[1a], LADEKAN YAYI C. Eléonore[1a,b], GBENOU Joachim D.[1a,2]

[1]*Department of Chemistry, Faculty of Sciences and Technology (FAST), University of Abomey-Calavi, Abomey-Calavi, Benin*
[a]*Laboratoire de Pharmacognosie et des Huiles Essentielles (LaPHE)*
[b]*Laboratoire de Chimie Organique Physique et de Synthèse (LaCOPS)*
[2]*Department of Chemistry, National University of Sciences, Technology, Engineering and Mathematics, Abomey, Benin*.

Abstract:
Epilepsy is a chronic neurological disease in developing countries. The access to appropriate care for the management of this disease being difficult in our countries, a large number of patients resort to the use of medicinal plants including Opilia celtidifolia. To contribute to the scientific knowledge of this plant and to promote the development of phytomedicines, we evaluated its antioxidant and anticonvulsant properties. The phytochemical screening of the aqueous extract of its leafy stems showed flavonoids (flavones), reducing compounds, anthocyanins, leuco-anthocyanins, saponosides, triterpenoids and mucilages. By spectrophotometric assays, total polyphenols and flavonoids were quantified. The extract was rich in total polyphenols and flavonoids with contents of 89.712 mg GAE/g extract and 37.040 mg QAE/g extract respectively. The antiradical activity was evaluated in vitro by DPPH test. The free radical scavenging activity gave an IC_{50} of 0.29 mg/mL. In a pharmacological model of epilepsy induced by pilocarpine (method developed and validated in Wistar rats), the administration of Opilia celtidifolia extract demonstrated the ability of this plant to significantly delay the onset of convulsions, to significantly reduce their intensity and to protect the rats from death precipitated by the violence of the convulsions. Finally, the larval toxicity of the extract was evaluated in vitro on Artemia salina and showed non-toxic activity (IC_{50}> 0.1 mg/mL). Although these preliminary results, obtained on the basis of animal experiments, are not immediately extrapolable to humans, they constitute a pharmacological argument in favor of the traditional use of the plant for the management of epilepsy.

Key Word*: Phytochemical screening, anticonvulsivant activity, antioxidant, toxicity, Opilia c.*

Date of Submission: 28-11-2022 Date of Acceptance: 17-12-2022

I. Introduction

At present, humanity is facing different kinds of diseases (microbial, parasitic, viral, cardiovascular, etc.) and the management of health issues is proving to be a real social problem, especially in developing countries [1,2]. According to an estimated research, about 80% of African populations use traditional medicines to meet their primary health care needs[3,4]. Thus, health care largely depends on medicinal plants and the local knowledge associated with them[3,5,6]. In traditional African environments, the use of plants against various diseases has been increasingly popular in recent years. This enthusiasm can be explained by cultural reasons, the decline in purchasing power, the high cost of conventional drugs and distrust of so-called modern synthetic products[7]. The search for new treatments accessible and available to the population is necessary and urgent. Medical plants are an ideal alternative (the best source) to chemical or special drugs that are too expensive to

manufacture or buy for developing countries[8]. This is how several plants are used not only for health care in traditional medicine but also for the search for new active molecules following one of the greatest challenges of global public health which is only the resistance of agents pathogens to antimicrobials[9]. It is therefore important to carry out ethnobotanical studies to identify the local uses of plant species[10,11].

In Benin, several ethnobotanical and ethnopharmacological studies are performed on medicinal species, their recipes as well as the forms of use by local populations[12-16]. Herbal drugs are thus prescribed empirically without any scientific knowledge of their biological activities and especially of the possible toxicity of their components [17]. It is in this context that plants whose therapeutic properties have been revealed in traditional medicine are the subject of in-depth pharmacological studies.

Nowadays, epilepsy is better understood and so-called conventional medicine offers effective treatments. It is a chronic neurological disease marked by synchronous and paroxysmal discharges of neurons. It is characterized by recurrent spontaneous seizures that can become complicated in status mal. From the neurological point of view, an imbalance is noted between excitatory neurons with glutamate or acetylcholine and inhibitory neurons with ɤ-amino butyric acid. The WHO estimates that around 50 million people suffer from epilepsy worldwide, 80% of whom live in developing countries[18]. Still in these countries, about 90% of people with the disease do not receive appropriate treatment, which explains why they remain marginalized and have a lower quality of life than other chronic patients[19]. However, contemporary medicine shows its limits: side effects, compliance difficulties and even resistance to treatment. For these reasons, some patients turn to complementary medicine, traditionnal medicine.

The prevalence of epilepsy in Benin is estimated at 8.05‰. Epilepsy increases the risk of premature death by about 2 to 3 times compared to the rest of the population [20]. The economic impact of epilepsy is high and estimated, according to an Indian study, at $344 per case per year. Recent studies have shown that 70% of epileptics can be successfully treated with antiepileptic drugs[18]. Nevertheless, ¾ of epileptic patients in developing countries do not have access to appropriate care. The majority of them therefore resort to the use of medicinal plants; but not all plants have been the subject of pharmacological study. This is what motivated the present study on *Opiliaceltidifolia* which thus caught our attention in order to contribute to its scientific knowledge and promote the development of phytomedicines.

The study aims to establish the phytochemical characteristics of the leafy stems of *Opilia celtidifolia*, to evaluate its toxicity, to develop an experimental convulsion model, to test the efficacy of the extract on validated experimental convulsions in Wistar rats.

II. Material And Methods

Material

The plant material consists of the leaves of *O. celtidifolia* which are harvested in the classified forest of Ahozon in Ouidah (Atlantic) and then dried at 20-25°C under the shelter of the sun in the laboratory for 3 weeks. They were then ground into powder using a grinding machine (Flour Gills NIG : EL. Motor N° 1827).

Figure 1: Aerial part of *O. celtidifolia*

The animal material consists of male and female Wistar rats weighing 130 to 220 g and brine shrimp larvae used for the larval toxicity test and for the anticonvulsant properties test respectively.

Figure 2 : Wistar rats

Figure 3 : Larvae *Artemia salina* L.

Methods

The phytochemical screening of the leafy stems was carried out according to the method of Houghton and Amala based on differential precipitation and coloring reactions[2b,12,21,22]. The extract used is a decoction obtained from the leafy stems of *O. celtidifolia*.

Preparation of the aqueous extract

The aqueous extract obtained by decoction of 50 g of *O. celtidifolia* leaf powder in 500 mL of distilled water for 30 min. The solution is left to decant for a few minutes, the mixture is filtered on Wathman paper and then the filtrate obtained was evaporated with rotavapor[2b,12,22].

Phytochemical screening

The qualitative chemical analysis was performed according to the method of Houghton and Raman[21] revised and adapted to the conditions of the Laboratory of Pharmacognosy and Essential Oils (LaPHE). It is based on differential precipitation and staining reactions[2,22]. The extract used is a decoctate obtained from the leafy stems of *O. celtidifolia*.

Determination of the antioxidant activity of the extracts

The antioxidant effect of the aqueous extracts of *O. celtidifolia* is determined by the DDPH method using 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH). Antioxidant activity is performed by the method of Lamien-Meda et *al.* [23]. The total antioxidant capacity of plant extract was determined by the methods of Hinneburg et *al.* [24] reported by Bakasso [25] and applied to our laboratory conditions[2b,12,22]. The positive control is represented by ascorbic acid (Vitamin C) which is treated under the same conditions as the tested samples.

The results were expressed as the average of three measurements. The IC_{50} value was determined for extract.

Toxicity test

The evaluation of the toxicity activity of our extract was performed on brine shrimp larvae using the adapted methods of Michael et *al*., Vanhaecke et *al*. and Mousseux [26-28]. This study consisted in determining the lethal concentration of our extract, at which 50% of the larvae were killed, and then in assessing its value.

Development of the experimental seizure model

The induction of convulsions for the development of our experimental model was carried out by the administration of pilocarpine nitrate i.p at 360 mg/kg. Note that a dose of 1 mg/kg of cholinergic antagonist, methyl scopolamine nitrate, was administered i.p., 30 min before that of pilocarpine to limit the peripheral effects of the latter.

Two groups of rats were formed for the validation of the model : a group representing epileptic rats (n = 9), having received scopolamine and pilocarpine and a control group representing non-epileptic rats ((n = 4), having received only distilled water.

Validation of the developed model

Seizure intensity was assessed using the six-score scale described by Racine [29]

- Score 0: no response,
- Score 1: seizures including hypoactivity and mouth and face automatisms,
- Score 2: convulsions including nodding and chewing,
- Score 3: convulsion including clonic movements of the front legs without rising,
- Score 4: seizures including clonic movements of the front legs and rising
- Score 5: rising and falling with loss of postural tone, general rigidity of the body (tonic-clonic seizure)

Scores were initially assigned every 15 min up to 90 min and then every 30 min until 180 min.

The research of the anticonvulsant activity of our extract was carried out in preventive treatment and in curative treatment.

Testing for anticonvulsant activity 1 (preventive treatment)

The preventive treatment consisted in the administration of the extract to the rats in i.p., before the induction of convulsions in i.e. 30 min before the administration of scopolamine. The extract was therefore administered to 3 different groups of rats at doses of 300, 400 and 500 mg/kg respectively. The 4th group received the reference drug, diazepam at 1 mg/kg in i.p.

The parameters considered to assess the preventive effect of the extract are : the intensity of the convulsions (in terms of its variation and the maximum score reached in rats) ; the latency time defined as the lapse of time separating the administration of pilocarpine from the appearance of clonic movements ; the time of attainment of status epilepticus (the latter being defined as uninterrupted or repeated convulsions of score 5 lasting at least 30 min) and then the mortality of the rats.

Testing for anticonvulsant activity 2 (curative treatment)

Curative treatment consisted of administration of the extract after induction of convulsions, i.e 15 min after administration of pilocarpine. The extract was therefore administered to 3 different groups of rats at doses of 500, 600 and 700 mg/kg respectively. The 4th group received Diazepam i.p. at a dose of 4 mg/kg.

The parameters considered to evaluate the curative effect of our extract are : intensity of the convulsions, ime to reach epilepticus status and the mortality of the rats.

Statistical analysis

Data were analyzed using STATISTICA Statsoft software version 5.5 and Microsoft Excel 2013. Student's t-test : latency. Analysis of variance from one-factor ANOVA decomposition test (tukey HSD post hoc test).The level $P < 0.05$ was considered as the cutoff value or significance.

III. Result

The present study was carried out in order to evaluate *in vitro* the anticonvulsant and antioxidant activity of the aqueous extract of this plant. Water was the solvent used not only because of its high extraction capacity, but also because it is the most widely used solvent in traditional medicine. Koffi et *al*.,[35] reported that the presence of water in plant organs would increase the permeability of plant tissues and promote the phenomenon of mass diffusion in the extraction step[36,37]. In Benin, it is practically the cheapest solvent, available to the whole population and less toxic compared to ethanol and other organic solvents. Even though ethanol is the second most used solvent by the population in traditional medicine, its use is sometimes limited and sometimes forbidden for some people because of their religious beliefs.

- ✓ Thus, we have privileged the aqueous extract in this work in order to valorize our results and to give an easy access to all traditional therapists for the exploitation of these results.

This aqueous extract obtained from the leaves of *O. celtidifolia* gave a good yield. Since the plant is widely used locally by traditional practitioners against several diseases, the qualitative chemical composition was evaluated for the extract prepared from its leafy stems.

After preparing the aqueous extract, several chemical and pharmacological analyses were studied in this work and the results are given below.

Phytochemical screening

The secondary metabolites present in the aqueous extract of the leafy stems of *O. celtidifolia* were detected by tube tests and the results are given in the following table 1 :

Table 1 : Phytochemical screening of *O. celtidifolia*

Chemical groups	**Aqueous extract**	**Chemical groups**	**Aqueous extract**
Alkaloids	-	Cardenolids	-
Catechic tannins	+/-	Cyanogenic derivatves	-
Gallic tannins	+/-	Mucilages	+
Flavones	+	Coumarins	+/-
Flavonols	-	Reducing compounds	+
Flavonones	-	Free anthracenics	-
Anthocyanins	+	Quinones derivatives	-

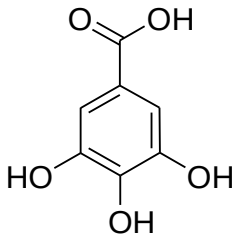
Leucoanthocyanins	+	O-heterosids	-
Saponosids	+	C-heterosids	-
Triterpenoids	+	Essential oil	n.d
Steroids	+/-	-	-

n.d. : not determined ; + : present ; - : absent ; +/- present but weakly (in trace)

Table no 1 shows phytochemical screening that revealed the presence of flavonoids (flavone), reducing compounds, anthocyanins, leucoanthocyanins, saponosides, triterpenoids and mucilages and the absence of alkaloids, cardenolides, cyanogenic derivatives, coumarins, free anthracenics, quinonic derivatives, O-heterosides and C-heterosides. Tannins and steroids were found in traces in the extract.

Determination (assay) of total polyphenols

The polyphenol content was determined using gallic acid as standard and the regression curve obtained with it.



Chemical structure of gallic acid

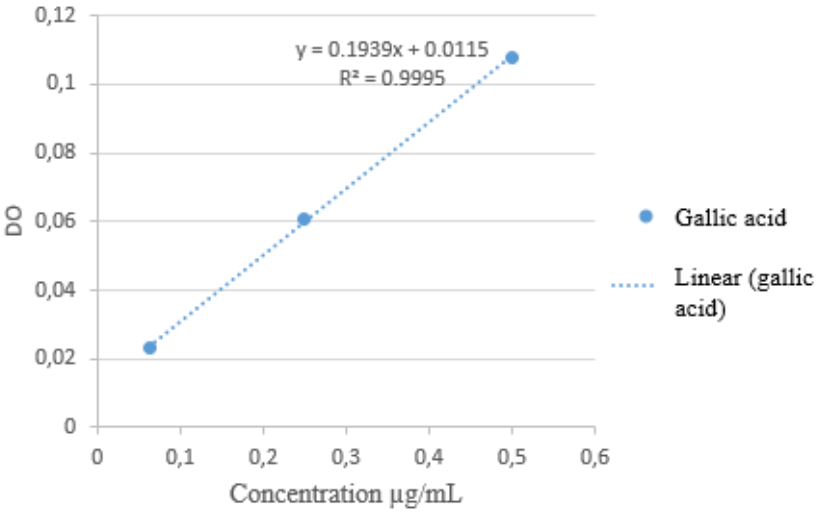


Figure 4 : Total polyphenol content of the aqueous extract of leafy stems

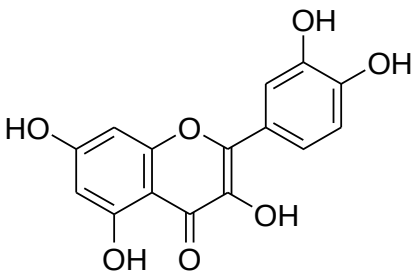
With the equation: y = 0.1939x + 0.0115, we have x = (0.374 - 0.0115)/0.1939 = 1.869.

$T = \frac{1.869 * Vr}{1 * 1}$ X 1000 , Vr = 6 mL.

The content of Total Polyphenols is 89.712 in mg of gallic acid equivalent/g of extract.

Determination (assay) of flavonoids

Quercetin was used as a standard for the determination of flavonoids content and the regression line is presented in Figure opposite 5.



Chemical structure of quercetin

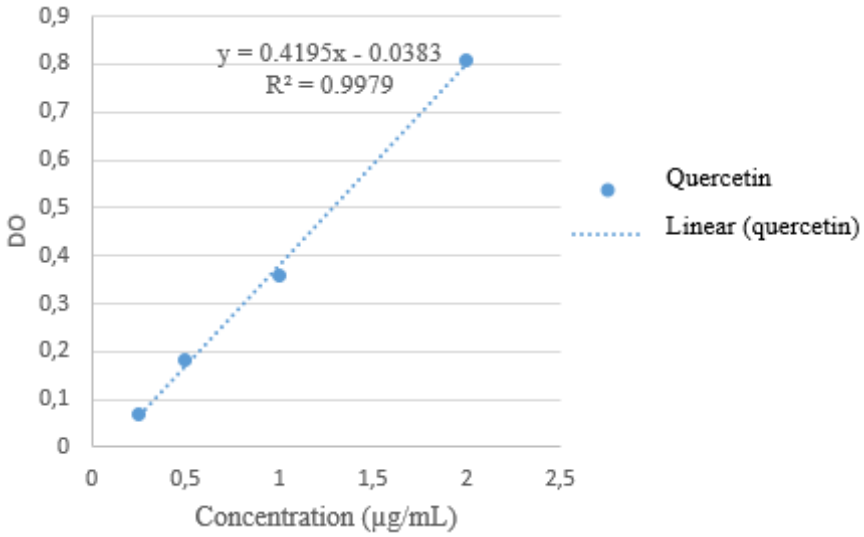


Figure 5: Flavonoid content of the aqueous leaf extract

With the equation y = 0.4195x – 0.0383 , we have :x = (1.904 + 0.0383)/0.4195 = 4.630.

$T = \frac{4.63 * Vr}{1 * 1}$ X 1000 ; Vr= 4 mL

The results revealed that the aqueous extract of leafy stems *O. celtidifolia* contains flavonoids in quantity with a content of 37.04 in mg quercetin equivalent /g extract.

Antioxidant activity of the extract

The antioxidant activity of the aqueous extracts by DPPH method is 0.29299 mg/mL for its IC_{50} and its percentage of inhibition (48.19%) at 0.33 mg /mL after that of ascorbic acid standard (98.19%) at the same concentration.

Characteristics of the developed model

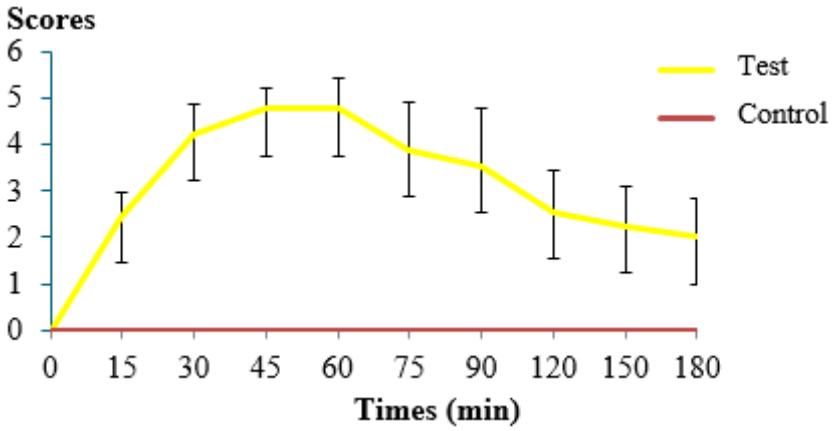


Figure 6 : Evolution of scores as a function of time in epileptic (test) and non-epileptic (control) rats.

From this figure, we can see that the seizure intensity of the developed model increased rapidly after pilocarpine administration to reach its maximum peak between 45th and 60th min, and then gradually decreased back to score 2 at 180th min.

Preventive effects of the extract on the evolution of the intensity of the convulsions

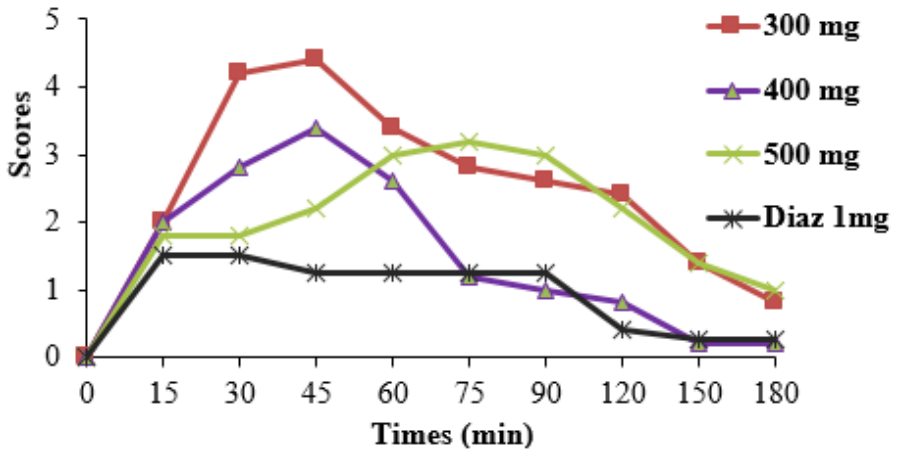


Figure 7 : Time course of seizure intensity during preventive treatment.

The figure showing the evolution of the intensity of the convulsions as a function of time showed us that the preventive treatment with the extract at the doses of 400 and 500 mg/kg and Diazepam at 1 mg/kg resulted in a statistically significant reduction of the intensity of the convulsions with a maximal effect for the extract at 400 mg/kg; Diazepam was more active than the extract on this parameter.

Preventive effects of the extract on the latency time

The diagramm showing the effect of the preventive treatment on the latency time presented that the extract, at doses of 400 and 500 mg/kg significantly delayed the onset of induced convulsions in rats with a maximal effect at the 500 mg/kg dose.

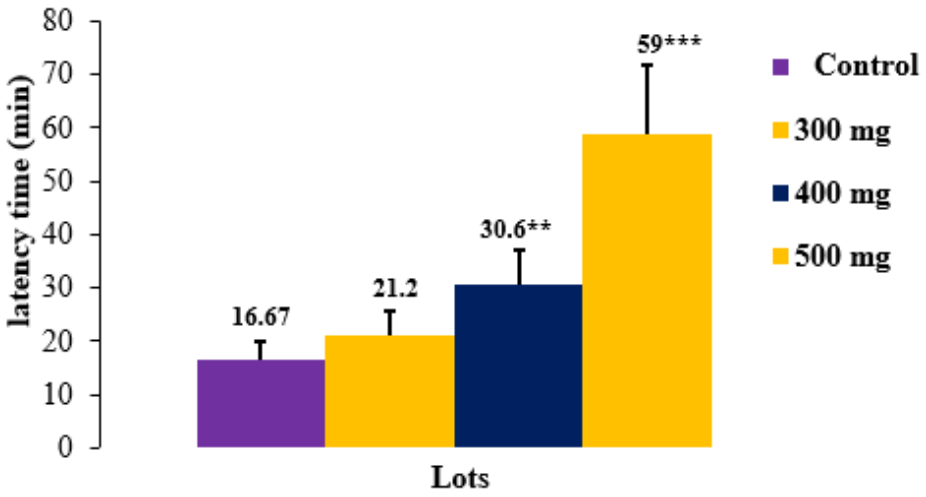


Figure 8 : Effect of preventive treatment on latency

Preventive effects of the extract on the maximum score achieved in the rat

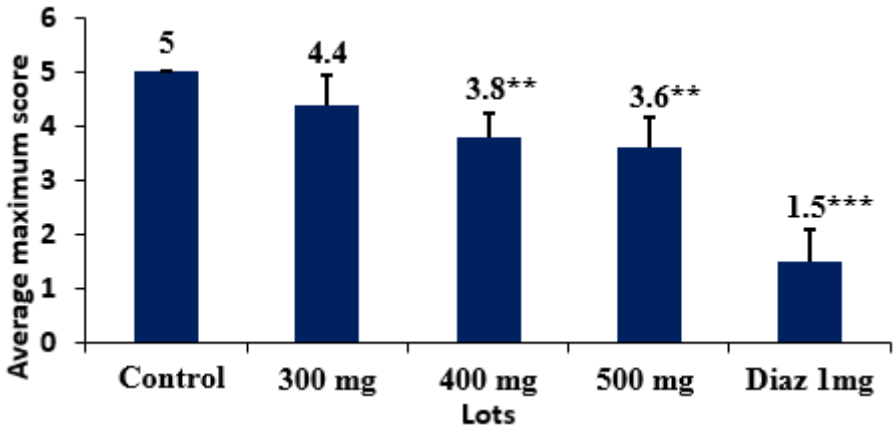


Figure 9 : Effect of preventive treatment on the maximum score achieved in rats

Our study showed that preventive treatment with the extract at 400 and 500 mg/kg resulted in a statistically significant reduction in the maximum score achieved in rats with a maximum effect at 500 mg/kg. Diazepam was also more active than the extract on this parameter.

Preventive effects of the extract on rat mortality

Table 2 : Rat mortality rate (preventive treatment)

Groups	Number of deaths	Mortality rate in %
Control (n = 9)	3	33.33
300 mg (n = 5)	1	20
400 mg (n = 5)	0	0
500 mg (n = 5)	0	0
Diaz 1 mg (n = 5)	0	0

The table showing the mortality rate of the rats after the preventive treatment reveals that the extract administered as a preventive measure at 400 and 500 mg/kg and Diazepam at 1 mg/kg prevented the death of the rats.

Table 3 : Rat mortality rate (curative treatment)

Groups	Number of deaths	Mortality rate in %
Control (n = 9)	3	33.33
E500 mg (n = 5)	1	20
E600 mg (n = 5)	1	20
E700 mg (n = 5)	1	20
Diaz 4 mg (n = 5)	0	0

The table showing rat mortality after curative treatment indicates that the curatively administered extract reduced rat mortality while Diazepam prevented rat death.

Toxicity activity

The toxicity test on *Artemia salina* L. gave the results expressed by the following figure :

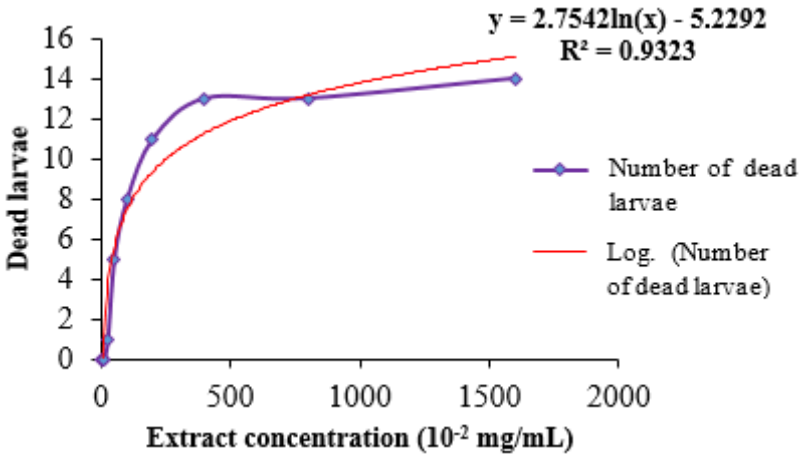


Figure 10 : Variation in larval mortality as a function of extract concentration

The results of the toxicity test revealed that the larval mortality respects a dose-response relationship. The logarithmic adjustment of order 2 (in red) of the curve expressing the variation of the mortality of the larvae according to the concentration of extract allowed us to determine the lethal half-concentration LC_{50} of our extract which is 1.22 mg/mL.

IV. Discussion

Choice of the plant Opilia celtidifolia and its aqueous extract.

O. celtidifolia is a plant well known by traditional health practitioners for the treatment of several diseases such as malaria, dermatosis, abdominal pain, as an aperitif, against wounds, intestinal worms etc. [30,31].

According to an ethnobotanical study conducted by Guinnin et *al*.,[3] it was noted that 04 plants are selected for further investigation because they are plants with more or less high frequency of citation on which very few studies are conducted. Among its plants, is *O. celtidifolia*. Concerning the various organs of the plants, the leaves (51.85%) are the organs most frequently used for medicinal preparations. These results are close to those of Sangare et *al*.,[32] who show that the leaves are solicited in majority during traditional treatments and differ from those of Thirumalai et *al*.,[33] who during a similar study in India discover that the most solicited part of the plant is the leafy stem. This state of affairs is understandable as the pathology of interest and the geographical area of study differ. The method of preparation of medicinal extracts varies according to the socio-cultural groups. Decoction (39.5%) is the most used method. This result is close to that established by Fah et *al*. who indicate that recipes are mainly prepared by decoction[34].

- ✓ These different observations and analyses justify our choice of the plant *Opilia celtidifolia* in this study.

The present study was carried out in order to evaluate *in vitro* the anticonvulsant and antioxidant activity of the aqueous extract of this plant. Water was the solvent used not only because of its high extraction capacity, but also because it is the most widely used solvent in traditional medicine. Koffi et *al*.,[35] reported that the presence of water in plant organs would increase the permeability of plant tissues and promote the phenomenon of mass diffusion in the extraction step[36,37]. In Benin, it is practically the cheapest solvent, available to the whole population and less toxic compared to ethanol and other organic solvents. Even though ethanol is the second most used solvent by the population in traditional medicine, its use is sometimes limited and sometimes forbidden for some people because of their religious beliefs.

- ✓ Thus, we have privileged the aqueous extract in this work in order to valorize our results and to give an easy access to all traditional therapists for the exploitation of these results.

These results, of phytochemical screening, partly corroborated those of researchers [38-45]. who reported the presence and/or absence of different chemical groups either in the powder or in the extract of the plant, depending on the techniques used. The presence of saponosides in the leaves and stems of O. celtidifolia was also confirmed in the work of Crespin et *al*.,[46]... The existence of polysaccharides was reported by Šutovská et *al*.,[47] who even isolated them from this plant. Some of these groups of compounds identified as coumarins,

flavonoids, triterpenes have pharmacological properties that could explain the possible anticonvulsant effect of our plant extract [48-51].

This variation in chemical composition could be explained by the conditions of harvesting and drying of leaves, the climate of the harvesting areas and the chemical composition of the soils, the time of harvesting of the plants during the day and the precision of the chemical tests used.

The presence of flavonoids indicates interesting pharmacological properties. Saponosides are well known for their surface active properties. These compounds can confer to the plant properties to decrease the permeability of blood capillaries and to reinforce their resistance [52].

If these different families of compounds could attribute to the plant various therapeutic properties which are quoted to him in traditional medicine, the polyphenols and in this case the flavonoïdes are not less quoted for their antioxydant activity. Thus, a quantitative analysis of these two large classes of compounds was carried out.

Antioxidant activity of the extract

This activity of the aqueous extract would be due to the presence of chemical families identified with antioxidant properties (Bruneton, 2008)[53] as polyphenols, flavonoids (flavones), anthocyanins, leucoanthocyanins, in the aqueous extract.

For a comparison study, ascorbic acid is used as a standard antioxidant and showed a very potent antiradical activity with an EC_{50} in the range of 0.2082 mg/mg DPPH, IC_{50} in the range of $1.11x10^{-2}$ mg/mL and PRA in the range of 480.307. The IC_{50} value of ascorbic acid is better than that previously reported (IC_{50} = 1.31 µg/mL) by Burits and Bucar [54].

The lowest free radical scavenging activity was expressed by the aqueous extract with EC_{50} = 5.497 mg/mg DPPH; IC_{50} = 0.292 mg/mL, and RPA of 5.497 which is 26 times lower than the reference. Ascorbic acid is then 26 times more active than the aqueous extract. The effect of the extract is most likely attributed to its richness in phenolic compounds and flavonoids.

According to Lee et *al*.,[53] any extract with an EC_{50} lower than 10 mg/mL is indeed an extract with antioxidant activity. In the case of our study, the EC_{50} of the aqueous extract is less than 10 mg/mL, which indicates that our extract has effective antioxidant activity.

Characteristics of the developed model

Our study (whit 360 mg/kg) gave a latency time (LT) of 16.67 ± 3.2 min and the time to status epilepticus (TSE) was 35.67 ± 13.33 min. Both times were higher than those obtained from the model developed by N'Gouemo («for 380 mg/kg, the LT = 10,1 ± 0,9 min » and the « TSE = 23 ± 2 min ») and could be explained by the reduced dose of pilocarpine in our study) [56].

At the dose of 360 mg/kg, we had an estimated 33.33% mortality of wistar rats. The mortality rate presented by the developed model is lower than that of the model developed by Jope et al. 57 with 400 mg/kg pilocarpine in Sprague Dawley rats (100% dead). This could be explained by the reduced dose of pilocarpine in our model, but also by the administration of scopolamine which limits the peripheral effects of pilocarpine. However, a different sensitivity linked to the species cannot be excluded.

Preventive effects of the extract of the intensity of the convulsions and on the latency time

Preventive treatment with the extract at 400 and 500 mg/kg and Diazepam at 1 mg/kg inhibited the development of pilocarpine-induced status epilepticus in rats.

None of the rats treated with Diazepam showed clonic movements. This indicates that diazepam is more active than the extract on this parameter.

The figure 9 shows that curative treatment with the extract at a dose of 700 mg/kg resulted in a statistically significant decrease in seizure intensity. Diazepam at 4 mg/kg was more active than the extract with a complete inhibition of convulsions as early as 90 min.

Curative treatment with both the extract and diazepam inhibited the development of pilocarpine-induced status epilepticus in rats.

In view of the above results, the dose of the extract that would offer a major preventive anticonvulsant effect would be between 400 and 500 mg/kg as it would both significantly decrease the intensity of convulsions

and significantly delay the onset of convulsions. Considering the effectiveness of the anticonvulsant activity of our extract, bioguided extractions deserve then to be undertaken for the relation chemical group and pharmacological effect. The reference drug, Diazepam was more active as a preventive, as well as a curative agent than the extract, all doses combined, whatever the parameter. The probable mechanisms of action of the anticonvulsant effect observed for this plant could concern an activation of the GABA receptors and/or an inhibition of the NMDA receptors, and therefore deserve to be explored.

This value, IC_{50} = 1.22 mg/mL; is higher than 0.1 mg/mL (threshold established by Mousseux[28] and above which the extract is considered not to be toxic). This allowed us to deduce that our aqueous extract used is not toxic on the larvae of *A. salina*. We can therefore say that our work has justified the traditional use of aqueous extracts of the plant *O. celtidifolia* by the population. However, further studies on the cytotoxicity of the extract will allow to better appreciate its effect on macrophage cells.

As mentioned recently by some researchers, there is a concordance between the biological activities of the chemical groups that were identified in our results with the traditional uses collected in previous ethnobotanical surveys [31,42].

V. Conclusion

The present study allowed to carry out phytochemical screening, quantification of polyphenolic compounds, antiradical activity, anticonvulsivant screening and larval toxicity of the aqueous extract of leafy stems of O. celtidifolia. The qualitative phytochemical studies revealed the presence of different families of compounds including polyphenols, flavonoids, mucilages etc. which could confer therapeutic properties to the plant. This extract presente high contents of polyphenols and flavonoids and the decoction of these leafy stems seems to be without toxicity. These results show then the interest of *O. celtidifolia* in the management of epilepsy and constitute prerequisites for the development of anticonvulsant phytomedicines. To promote and enhance the development of improved traditional medicines.

Acknowledgments

The authors would like to thank all the researchers from our laboratories who participated in the work.

Ethical Issues

Not applicable.

Conflict of Interest

The authors declare that there is no conflict of interest

References

[1]. Mangambu MJD, Mushagalusa KF, Kadima NJ. Contribution á l'étude phytochimique de quelques plantes médicinales antidiabétiques de Bukavu, R.D Congo. Journal of Applied Biosciences 2014;75:6211– 6220. http://dx.doi.org/10.4314/jab.v75i1.7

[2]. (a) Burkill HM. The Useful plants of West Tropical Africa. 2nd Edition, Volume 4. Royal Botanic Garden, kew, 1997. (b)Toklo PM, Ladekan-Yayi EC, Sakirigui A, Alowanou GG, Assogba MF, Challaton KP, Hounzangbe-Adote S, Gbenou JD. Antioxidant and antiparasitic activities on Trichostrongylus colubriformis of the aqueous extract of fresh leaves of Mitragyna inermis (Willd.) Kuntze (Rubiaceae). The Pharmaceutical and Chemical Journal 2021; 8(1):120-128.

[3]. Guinnin FDF. Sacramento TI, Sezan A, Ategbo J-M. Etude Ethnobotanique des plantes médicinales utilisées dans le traitement traditionnel des hépatites virales B et C dans quelques départements du Bénin. Int J Biol Chem Sci 2015;9(3):1354-1366. Doi : http://dx.doi.org/10.4314/ijbcs.v9i3.20

[4]. WHO. World Health Report : Reducing Risks, Promoting Healthy Life. Geneva: World Health Organization 2002;7-14. https://apps.who.int/iris/handle/10665/42510

[5]. Fyhrquist P. Traditional medecinal uses and biological activities of some plants extract of African Combretum Loefl; Terminalia L. and Pteleopsis Engl. Species (Combretaceae). Ph.D. Dissertation, University of Helsinki, Finland, 2007, p.185.

[6]. Sinsin B, Tèhou AC, Daouda I, Saidou A. Abudance and species richness of large mammals in Pendjari National Park in Bénin. Mammalia 2002;66:369-380.

[7]. Nwakile DC, Okore VC. Picralima nitida Seed Oil I: Hypoglycemic Activity. Journal of Advanced Pharmacy Education & Research 2011;2:147-153.

[8]. Doughari JH, El-Mahmood AM and Manzara S. Studies on the Antibacterial Activity of Root Extracts of Carica papaya L. African Journal of Microbiology Research 2007;1:37-41. doi : 10.4236/aim.2017.76036

[9]. Mezouar S, Mege D, Darbousset R, Farge D, Debourdeau P, Dignat-George F, Panicot-Dubois L, Dubois C. Involvement of Platelet-Derived Microparticles in Tumor Progression and Thrombosis. Seminar on Oncology 2014;41(3):346-358. doi: 10.1053/j.seminoncol.2014.04.010.

[10]. Betti JL. An ethnobotanical study of medecinal plants among the Baka Pygmies in the Dja Biosphere reserve (Cameroon). Afr. Stud Monogr 2004;25(1):1-27. DOI:10.14989/68229

[11]. Betti JL. Usages traditionnels et vulnérabilité des plantes médicinales dans la réserve de biosphère du Dja, Cameroun. Thèse de Doctorat, Université Libre de Bruxelles, Belgique, 2001, p.87.

[12]. Yomakou Bato Georges GR, Glinma B, Assogba MF, Akakpo BH, Ahoton D, Aïkpe AJF, Yayi Ladekan E, Gbenou JD. Phytochemistry, metabolites quantification and antioxidant activity of Calotropis procera (Ait.) and Ficus umbellata (Vahl.), plants traditionally used against hemorrhoids in Benin. Int J Curr Res Chem Pharm Sci 2021;8(12):12-26. doi: http://dx.doi.org/10.22192/ijcrcps.2021.08.12.002

[13]. Ahomadegbe MA, Toklo PM, Glinma B, Kakpo BA, Agbani PO, Assogba FM, Yayi Ladekan E, Gbenou JD. Plants used against diarrheal diseases in traditional African medicine: crossreferencing, pharmaco-chemical for a valorization pedagogical perspectiv. Chemistry Research Journal 2021;6(6): 89-110.

[14]. Déléké Koko I, Djégou J, Gbénou J, Hounzangbé-Adoté SM, Sinsin B. Etude phytochimique des principales plantes galactogènes et emménagogues utilisées dans les terroirs riverains de la zone cynégétique de la Pendjari. Int J Biol Chem Sci 2011;5(2):618-633.

[15]. Bieke B. Etnobotanish studie van geneeskrachtige platen in manigri en Igbre, Benin. Universtieit Gent., Bioingenieurin hetland, En Bos Beheer, 2004, 420p.

[16]. Sokpon N, Ouinsavi C. Utilisation du Khaya senegalensis en médecine traditionnelle au Bénin. Revue Méd Pharm Afr 2002;16:9-19.

[17]. Gupta A, Khamkar PR, Chaphalkar S. Applications and Uses of Active Ingredients from Medicinal Plants. Indian Journal of Novel Drug delivery 2014;6(2):106-111.

[18]. WHO. Epilepsy. 9 february 2022. https://www.who.int/news-room/fact-sheets/detail/epilepsy.

[19]. OMS. Epilepsy: a public health imperative. Geneve 2019. https://www.who.int/publications/i/item/epilepsy-a-public-health-imperative

[20]. Agbetou M, Kabibahou H, sowanou A, Kossi O, Houehanou C, Adoukonou TA. Knowledge, attitudes and practices of a semi-urban population on epilepsy: case of djougou in benin in 2019. African Journal of Neurological Sciences. 2021;40(2):72-78. https://www.ajol.info/index.php/ajns/article/view/231963

[21]. Houghton PJ, Raman A. Laboratory handbook for the fractionation of natural extracts. New York: Chapman and hall. 1998; p199.doi:10.1007/978-1-4615-5809-5.

[22]. Assogba MF. Phytochimie et propriétés pharmacobiologiques des extraits de feuilles de Elaeis guineensis Jacq (Arecaceae). Thèse de Doctorat Unique, Université d'Abomey-Calavi, 2016.

[23]. Lamien-Meda A, Lamien CE, Compaoré MMY, Meda RNT, Kiendrebeogo M, Zeba B, Millogo JF, Nacoulma OG. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules 2008;13(3):581-94. doi:10.3390/molecules13030581.

[24]. Hinneburg I, Damien DHJ, Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem. 2006;97:122–129. https://doi.org/10.1016/j.foodchem.2005.03.028

[25]. Bakasso S. Etudes phytochimiques et potentialités biologiques de cinq espèces d'indigofera (fabaceae) utilisées en médecine traditionnelle au Burkina Faso. Doctorat Es Sciences Biologiques Appliquées, 2009, Université de Ouagadougou.

[26]. Michael AS, Thompson CG, Abramovitz M. Artemia salina as a test organism for bioassay. Science 1956;123(3194):464. doi: 10.1126/science.123.3194.464.a

[27]. Vanhaecke P, Persoone G, Claus C, Sorgeloos P. "Proposal for a short-term toxicity test with Artemia nauplii". Ecotoxicol. Environ. Safety 1981;5(3):382-387. https://doi.org/10.1016/0147-6513(81)90012-9.

[28]. Mousseux M. Test de toxicité sur les larves d'Artemia salina entretien d'élevage de balanes. Université française de Pacifique. Centre universitaire de Nouvelle Calédonie. Université Française du Pacifique. Mém. Duest. Aquaculture 1995, p.20.

[29]. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure Electroencephalogr Clin Neurophysiol, 1972;32(3):281-294. DOI: 10.1016/0013-4694(72)90177-0

[30]. Karabinta ADK. Propriété cicatrisante des feuilles de Opilia celtidifolia (Guill. et Perr.) Endl. ex Walp. (Opiliaceae). Thèse d'Etat en Pharmacie, Université de Bamako, Mali. 2010.

[31]. Togola A, Diallo D, Dembélé S, Barsett H, Paulsen BS. Ethnopharmacological survey of different uses of seven medicinal plants from Mali, (West Africa) in the regions Doila, Kolokani and Siby. Journal of Ethnobiology and Ethnomedicine 2005;1:7. https://doi.org/10.1186/1746-4269-1-7

[32]. Sangare MM, Sina H, Dougnon J, Bayala B, Ategbo JM, Dramane KL. Etude ethnobotanique des plantes hépatotropes et de l'usage traditionnel de Gomphrena celosioides Mart. (Amaranthaceae) au Bénin. Int J Biol Chem Sci 2012;6(6):5008-5021. doi: 10.4314/ijbcs.v6i6.20

[33]. Thirumalai T, Beverly CD, Sathiyaraj K, Senthilkumar B, David E. Ethnobotanical Study of anti-diabetic medicinal plants used by the local people in javadhu hills Tamilnadu, India. Asian Pacific Journal of Tropical Biomedicine 2012;2(2):S910-S913. https://doi.org/10.1016/S2221-1691(12)60335-9

[34]. Fah L, Klotoé JR, Dougnon V, Koudokpon H, Fanou VBA, Dandjesso C, Loko F. Etude ethnobotanique des plantes utilisées dans le traitement du diabète chez les femmes enceintes à Cotonou et à Abomey-Calavi. Journal of Animal & Plant Sciences 2013;18(1):2647-2658.

[35]. Koffi AG, Ahoua ARC, Ekou L, Ekou T, Kone MW. Activité antioxydante de quelques plantes utilisées dans la région de Tiassalé (Côte d'Ivoire) dans le maintien de la santé de la peau. European Scientific Journal 2018;14:30.338. DOI: https://doi.org/10.19044/esj.2018.v14n30p338

[36]. Durant M, Molinier V, Kunz W, Aubry J-M. Classification of organic solvents revisited by using the COSMO-RS approach. Chemistry – A European Journal 2011;17(18):5155-5164. https://doi.org/10.1002/chem.201001743

[37]. Moure A, Franco D, Sineiro J, Dominguez H, Nunez MJ, Lema JM. Evaluation of extracts from Gevuina avellana hulls as antioxidants. Journal of Agricultural and Food Chemistry 2000;48(9):3897-3890. https://doi.org/10.1021/jf000048w

[38]. Koumare B, Diallo D, Sanogo R, Diarra B, Doumbia S, Diarra M, Soumare M. Study of Phytochemistry and Appetizing Activity of Decocted Leaves of Opilia celtidifolia Guill. and Perr. (Opiliaceae) in Rats. EasyChair Preprint 2020;№ 3407:17.

[39]. Amang AP, Kodji E, Mezui C, Baane MP, Siwe GT, Kuissu TM, Emakoua J, Tan PV.. Hepatoprotective Effects of Aqueous Extract of Opilia celtidifolia (Opiliaceae) Leaves against Ethanol-Induced Liver Damage in Rats. Evidence-Based Complementary and Alternative Medicine 2020;1-8. https://doi.org/10.1155/2020/6297475

[40]. Mazadu EA, Misau MS, Gwallameji LB. Phytochemical screening and antimicrobial activity of some medicinal trees grown in Bauchi state, north eastern, Nigeria. Journal of Pharmacognosy and Phytochemistry 2018;7(2):3503-3507.

[41]. Owolabi MS, Omowonuola AA, Lawal OA, Labunmi L, Dosoky NS, Collins JT, Ogungbe IV, Setzer WN. Phytochemical and bioactivity screening of six Nigerian medicinal plants. Journal of Pharmacognosy and Phytochemistry. 2017;6(6):1430-1437.

[42]. Togola A, Karabinta K, Dénou A, Haidara M, Sanogo R, Diallo D. Effet protecteur des feuilles de Opilia celtidifolia contre l'ulcère induit par l'éthanol chez le rat. Int J Biol Chem Sci 2014;8(6):2416-2423. DOI: 10.4314/ijbcs.v8i6.5

[43]. Koudouvo K, Karou SD, Ilboudo DP, Kokou K, Essien K, Aklikokou K, de Souza C, Simpore J, Gbéassor M. In vitro antiplasmodial activity of crude extracts from Togolese medicinal plants. Asian Pacific Journal of Tropical Medicine 2011;129-132. doi:10.1016/S1995-7645(11)60052-7
[44]. Karabinta ADK. Propriété cicatrisante des feuilles de Opilia celtidifolia (Guill. et Perr.) Endl. ex Walp. (Opiliaceae). Thèse de doctorat à la Faculté de Médecine de Pharmacie et d'Odonto-Stomatologie de l'Université de Bamako. 2010.
[45]. Sangaré D. Etude de la prise en charge du paludisme par les thérapeutes traditionnels dans les aires de santé de Kendie (Bandiagara) et de Finkolo (Sikasso). Thèse de Pharmacie, Université de Bamako. 2003; p131.
[46]. Crespin F, Olliver E, Lavaud C, Babadjamian A, Faure R, Debrauwer L, Balansard G, Boudon G. Triterpenoid saponins from O. celtidifolia. Phytochemistry 1993;33(3):657-661. https://doi.org/10.1016/0031-9422(93)85468-7
[47]. Šutovská M, Fraňová S, Prisežnaková L, Nosáľová G, Togola A, Diallo D, Paulsen BS, Capek P. Antitussive activity of polysaccharides isolated from the Malian medicinal plants. International Journal of Biological Macromol 2009;44 (3):236-239. https://doi.org/10.1016/j.ijbiomac.2008.12.013
[48]. Bruneton J. Pharmacognosie, phytochimie, plantes médicinales. 3e édidion. Paris: Technique et Documentation Lavoisier. 2009.
[49]. Hua OH, Tran QTT, Trinh D-TT, Nguyen V-D, Duong DPN, Nguyen TT. A Review of Traditional Uses, Phytochemistry and Pharmacological Properties of Some Vietnamese Wound-Healing Medicinal Plants. Natural Product Communications 2022;17(4). doi:10.1177/1934578X221088379.
[50]. Jäger AK, Saaby L. Flavonoids and the CNS. Molecules 2011;16(2):1471-1485. https://doi.org/10.3390/molecules16021471.
[51]. Kasture VS, Chopde CT, Deshmukh VK. Anticonvulsive activity of Albizzia lebbeck, Hibiscus rosa sinesis and Butea monosperma in experimental animals. Journal of Ethnopharmacology 2000;71(1-2):65-75. https://doi.org/10.1016/S0378-8741(99)00192-0.
[52]. Bruneton J. Pharmacognosie Phytochimie Plantes médicinales, Edition Technique et Documentation Lavoisier, Paris, 1993;387-404.
[53]. Bruneton J. Pharmacognosy Phytochemistry Medicinal plants 2nd Edition. Tec Et Doc EAN : 2008;9782743010584.
[54]. Burits M, and Bucar F. Antioxidant Activity of Nigella sativa Essential Oil. Phytotherapy Research 2000;14(5):323-328. https://doi.org/10.1002/1099-1573(200008)
[55]. Lee YL, Yang JH, Mau JL. Antioxidant properties of water extracts from Monascus fermented soybeans. Food Chem 2008;106(3):1128-37. https://doi.org/10.1016/j.foodchem.2007.07.047.
[56]. N'Gouemo P, Baldy-Moulinier M, Nguemby-Bina C. Some pharmacological effects of an ethanolic extract of Palisota ambigua on the central nervous system in mice. Phytotherapy Research 1994;8(7):426-429. https://doi.org/10.1002/ptr.2650080710.
[57]. Jope RS, Morrisett RA, Snead OC. Characterization of lithium potentiation of pilocarpine-induced status epilepticus in rats. Experimental Neurology 1986;91(3):471-480. https://doi.org/10.1016/0014-4886(86)90045-2.

DASSOU E. Morel, et. al. "Phytochemical study, antioxidant and anticonvulsant activities of aqueous extract of leaves of Opilia celtidifolia (guill. Et perr.) Endl. Ex walp. Opiliaceae, from Benin." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 17(6), (2022): pp. 43-55.