IN VIVO NEUROPROTECTIVE ACTIVITY OF THE CHLOROFORM SELECTIVE EXTRACT FROM THE BARK OF *ERYTHRINA SENEGALENSIS* DC. (FABACEAE) AND CHARACTERIZATION OF TWO ISOLATED PHYTOCONSTITUENTS

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ABSTRACT

Marcellin, A.K., Kadja, A., Akhanovna, M-B. J., Tangui M., David V., Jean-Luc, P., Pascale, G., and Yves-Alain, B. (2022). *In vivo* Neuroprotective Activity of the Chloroform Selective Extract from the Bark of *Erythrina senegalensis* DC. (Fabaceae) and Characterization of two Isolated Phytoconstituents. *Lebanese Science Journal,* 23(2): 146-162.

The present study aims to isolate and identify bioactive phytocompounds derived from the crude methanolic extract, an effective extract of Erythrina senegalensis DC. (Fabaceae). The chloroform extract (selective extract of the crude methanol extract) was obtained by liquid-liquid extraction, then tested for its protective activity in vivo against Alzheimer's disease in a mouse model. After showing a beneficial effect, this extract underwent chromatographic (open column, HPLC) and spectroscopic (NMR) analyzes in order to isolate and characterize the phytocompounds which would be at the origin of the observed biological activities. Thus, two phycompounds of the flavonoid class: osajin and cajaflavanone, were isolated for the first time from E. senegalensis from Côte d'Ivoire.

Keywords: Alzheimer's disease, Cajaflavanone, *Erythrina senegalensis* DC (Fabaceae), HPLC, NMR, Osajin.

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INTRODUCTION

Erythrina senegalensis DC (Fabaceae) is an ornamental, medicinal plant widely used by the populations of West Africa (Adjanohoun et al., 1986). The different parts of the plant are used in the treatment of many diseases such as hematuria, rheumatism, jaundice, malaria, female infertility, fibroids, etc. (Iwu, 1993; Nacoulma, 1996; Togola et al., 2008). In Côte d'Ivoire, ethnopharmacological surveys show that extracts from the bark of the trunk are also used in the treatment of mental disorders. Thus, the work carried out by Ahué et al., 2017 made it possible to show the neuroprotective effects of the methanol extract of the bark of said plant. These authors also observed, among the various selective extracts, a better antioxidant profile with respect to the DPPH radical of the chloroform extract. The main objective of this work would therefore be to test the effect of this selective extract on a mouse model of Alzheimer's disease, and to isolate and characterize the phytocompounds there by HPLC. -UV and 1H and 13C NMR.

MATERIAL AND METHODS

Study extract

The chloroform extract noted E2, derived from the methanol extract of the bark of *Erythrina senegalensis* (Ahué et al., 2017) was the support of this study.

Methods

A- Evaluation of the *in vivo* Neuroprotective Activity of E2

The evaluation of the neuroprotective potential of E2 was carried out in mice after administration of the Aβ25-35 peptide (Ahué et al., 2017).

B- Drugs and Administration Procedures

The Sc.Aβ (non-toxic control) and $A\beta_{25-35}$ (toxic) peptides were solubilized in sterile distilled water at 3 mg/mL and stored at -20°C. Peptides were incubated at 37°C for 4 days in order to induce their oligomerization (Maurice et al., 1996; Zussy et al., 2011). $A\beta_{25-35}$ or Sc.Aβ (9 nmol/mouse) were then injected intracerebroventricularly in a final volume of 3 µL/mouse (Maurice et al., 1996).

The chloroform extract was solubilized in DMSO 10% in water (vehicle solution V2) to provide a stock solution (40mg/mL). From the 1st to the 7th day of the experiment, V2 or the extract (**Table 1**) was administered to the mice orally (100 µL/20 g of body weight) as a preventive treatment. Peptides were injected on the 8th day. The administration of V2 or the extract continued for a week after injection of the curative peptides. The acetylcholinesterase inhibitor Donepezil was used as a reference drug in a separate set of experiments. The drug was solubilized in water (vehicle solution V1) and administered at 1 mg/kg intraperitoneally (IP)o.d. for a week after peptide injection.

Table 1. PO injection preparation method

Peptide	Extract	Number of mice
1. ScAβ25-35(non-toxic)	V2	12
2. Aβ25-35 (toxic)	V2	12
3. Aß25-35(toxic)	Studyextract(E2)	12
Total number of mice		**36**

Abbreviations: V2, vehicle solution: distilled water/DMSO 90/10; Sc.Aβ, scrambled $Aβ_{25-35}$ peptide; PO, per os.

C- Behavioral Test and Biochemical Analysis

The animals were treated with strict respect for bioethics.

✓ ***Spontaneous Alternation Test in the Y-Maze***

On the 7th day after injection of the peptides, all animals were tested for performance of spontaneous alternation in the Y-maze, which measures working spatial memory (Maurice et al., 1996).

✓ ***Passive Avoidance Test***

The long-term contextual memory in mice was assessed using a passive avoidance procedure, with the training session on the 8th day after the peptide injection and the retention session on the 9th day (Rodriguez Cruz et al., 2017).

✓ ***Measurement of lipid peroxidation levels in the mouse hippocampi***

For biochemical analysis purposes, the animals were sacrificed (under deep ketamine/xylazine anesthesia) 10 days after peptide injection by decapitation. The hippocampus was extracted, flash frozen, and used for biochemical assay measuring the level of peroxidation of the membrane lipids as previously described (Rodriguez Cruz et al., 2017).

D- Statistical Analyses

The results were analyzed by an ANOVA followed by a Dunnett's multiple comparison test. Passive avoidance data were non-parametric since a maximum value was imposed. They were expressed as median and interquartile 25%-75% range. They were analyzed using Kruskal-Wallis nonparametric ANOVA, followed by a Dunn's multiple comparison test. The level of significance was $p < 0.05$. Statistical values are detailed in the figure legends.

E- Extraction and Structural Determination of Isolated Phytoconstituents

Isolation of Phytoconstituents

5.6 g of E2 dissolved in CH_2Cl_2 were introduced into an open column containing SdS silica gel (granulite 35-70 µm, 72 g), prepared in CH_2Cl_2 (Traoré et al., 2017).
The elution was carried out with a gradient of three solvents ($PhCH_3$ / $CHCl_3$ / AcOEt) by setting the volume of the first two, and gradually varying the last (15/10; 15/10/2; 15/10/4; 15/10/6; 15/10/8; 15/10/10 (*v/v/v*)). The fractions collected were analyzed by TLC in order to group those that show a similar chromatographic profile at the wavelengths 254 nm and 365 nm. These were combined and the eluent was removed by evaporation under vacuum.

Four fractions E21 (0.78 g), E22 (1.37 g), E23 (1.08 g), and E24 (1.23 g) were obtained and were again fractionated by column chromatography. The fraction E22, showing fewer spots on its chromatographic profile, was fractionated with a gradient of solvents Hexane/AcOEt (95/5; 90/10; 85/15; 80/20; 75/25; 70/30; 65/35) and yielded sub-fraction D1 (1.2 mg), D2 (78 mg) and D3 (523 mg). The sub-fraction D2 was retained for the various spectral analyses.

Structural Determination of Isolated Phytoconstituents

The analysis was carried out on a Shimadzu Varian 400MHz HPLC apparatus fitted with a UV- Visible D107 detector, a Rainin Dynamax AI-1A type Injector fitted with a Gemini C18 110 R5µ column, 100x 4.6 mm in reverse phase with particles 1.7 µm in diameter. The dry extract was dissolved in H_2O/MeCN (10/90; 1 mg/mL), filtered on a micro sinter, and then injected. The flow rate of the mobile phase was 1mL/min. The data was retrieved and analyzed by the LC solution software.
For the NMR analysis (Shimadzu 400 MHz Varian 5 mm), after drying the sample with the vane pump, 30 mg were taken and solubilized in 2 mL of $CDCl_3$ then 1 mL was analyzed. The data was processed with the MestReNOVA.

RESULTS AND DISCUSSION

Behavioral and Biochemical Analyses

The intracerebroventricular administration of $A\beta_{25-35}$ in mice causes memory deficits and decreases the activity of choline acetyltransferase. Therefore, the toxicity model of $A\beta_{25-35}$ is a pertinent *in vivo* drug screening model. Positive control animals were injected with a reverse control, nontoxic, peptide composed of the amino-acids of the [25-35] fragment in a random sequence. The ICV injection of $A\beta_{25-35}$ caused significant alterations in mice after one week. The body weight of the animals was checked daily during the period of injection of the extract. Administration of the E2 extract did not affect body weight as compared to the groups treated with Sc.Aβ/V2 orSc.Aβ/V1 (data not shown).

- ✓ ***Spontaneous Alternation Test in the Y-maze***

The $A\beta_{25-35}$ treatment induced a significant deficit of spontaneous alternation compared to the Sc.Aβ-injected mice (Figure 1). Sc.Aβ mice showed an alternation performance around 68%, thus above the chance level of 50%, confirming that the mice used their spatial working memory to explore the Y-maze. $A\beta_{25-35}$-injected mice showed a significant alternation deficit (Figures 1a and 1b). These deficits were attenuated by the E2 extract, since the $A\beta_{25-35}$-/E2-treated group did not show a significant difference with the control Sc.Aβ/V2 group (Figure 1a). As a comparator, donepezil completely and significantly blocked the deficit in spontaneous alternation induced by $A\beta_{25-35}$ (Figure 1b). This experiment suggested a moderate protective effect of the E2 extract on a parameter related to working memory alteration in $A\beta_{25-35}$ mice.

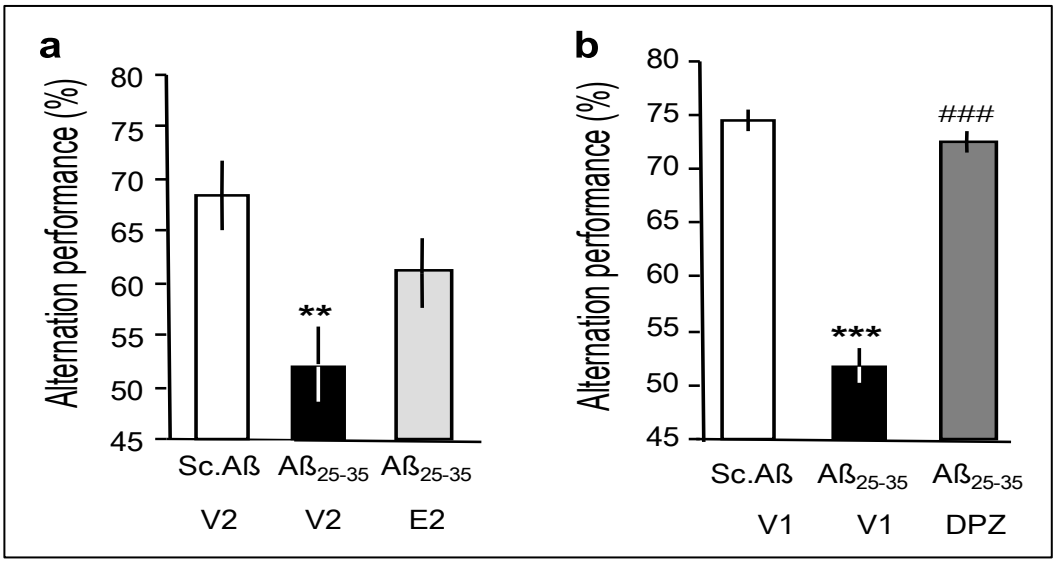

Figure 1. Protective effects of the E2 extract and of donepezil on the Aβ25-35-induced spontaneous alternation deficits in mice. Abbreviations: Sc.Aβ, scrambled peptide; V1, vehicle solution 1 (distilled water); V2, vehicle solution 2 (distilled water/DMSO 90/10); DPZ, donepezil; E2, chloroform selective *Erythrina senegalensis* extract. ANOVA: $F_{(2,35)} = 5.64$, $p < 0.01$, n = 11-13 per group in (a); $F_{(2,35)} = 103.5$, $p < 0.0001$, n = 12 per group in (b). ** P < 0.01 vs. (Sc.Aß/V) group; ### P < 0.001 vs. (Aβ25-35/V) group; Student's *t*-test.

- ✓ ***Passive Avoidance Test***

The $A\beta_{25-35}$ treatment induced a significant step-through latency deficit in the passive avoidance response measured during the retention session, as compared to Sc.Aβ-injected mice (Figure 2). Note that in our experiments, the $A\beta_{25-35}$ effect slightly differed in intensity between the two experiments, testing E2 extract (median value 34 s; Figure 2a) and donepezil (median value 136 s; Figure 2b). The administration of E2 extract significantly increased the step-through latency in $A\beta_{25-35}$ treated mice up to the median value of the control group (150 s; Figure 2a). Similarly, the donepezil treatment resulted in a significant increase in the step-through latency up to the median value shown by the control group (255 s; Figure 2b). These results showed that E2 extracts completely prevented $A\beta_{25-35}$-induced deficits, as observed with the reference drug.

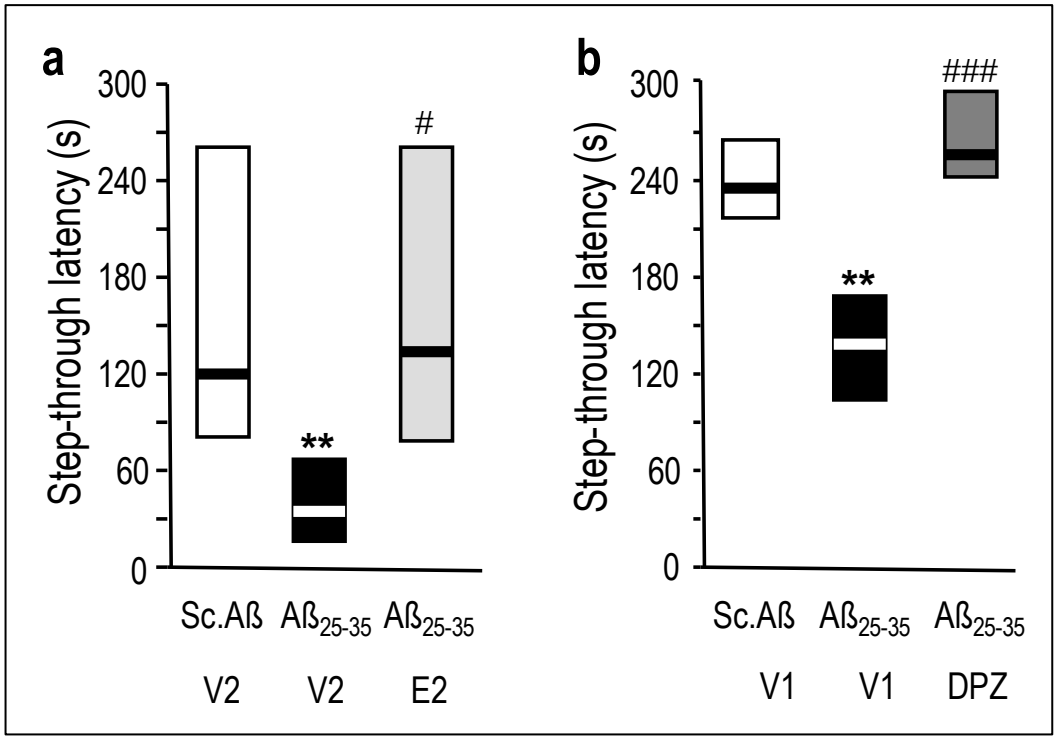

Figure 2. Protective effects of the E2 extract and donepezil on the Aβ$_{25-35}$-induced passive avoidance deficits in mice. Abbreviations: Sc.Aβ, scrambled peptide; V1, vehicle solution 1 (distilled water); V2, vehicle solution 2 (distilled water/DMSO 90/10), DPZ, Donepezil; E2, chloroform selective *Erythrina senegalensis* extract. Kruskal-Wallis ANOVA: H = 12.0, p < 0.01, n = 11-16 per group in (a); H = 21.2, p < 0.0001; n = 12 per group in (b). ** P < 0.01 vs. (Sc.Aß/V) group; # P < 0.05, ### P < 0.001 vs.(Aβ$_{25-35}$/V) group; Student's *t*-test.

✓ ***Measurement of lipid peroxidation levels (oxidative stress) in the mouse hippocampus***

Oxidative stress is a major component of the toxicity observed in Alzheimer's patient brains and responsible for neuroinflammation and synaptic failure, thus leading to cognitive deficits. The Aβ$_{25-35}$ treatment induced a significant increase in lipid peroxidation level in the hippocampus as compared to Sc.Aβ-injected mice that varied from +19% in the E2 experiment (Figure 3a) to +65% in the donepezil experiment (Figure 3b). The administration of E2 extract or donepezil completely prevented the increase in lipid peroxidation (Figures 3a and 3b). These biochemical results confirmed the behavioral observations by showing that E2 extract was effective in preventing Aβ$_{25-35}$-induced toxicity in mice, in a comparable manner as observed with the reference drug donepezil.

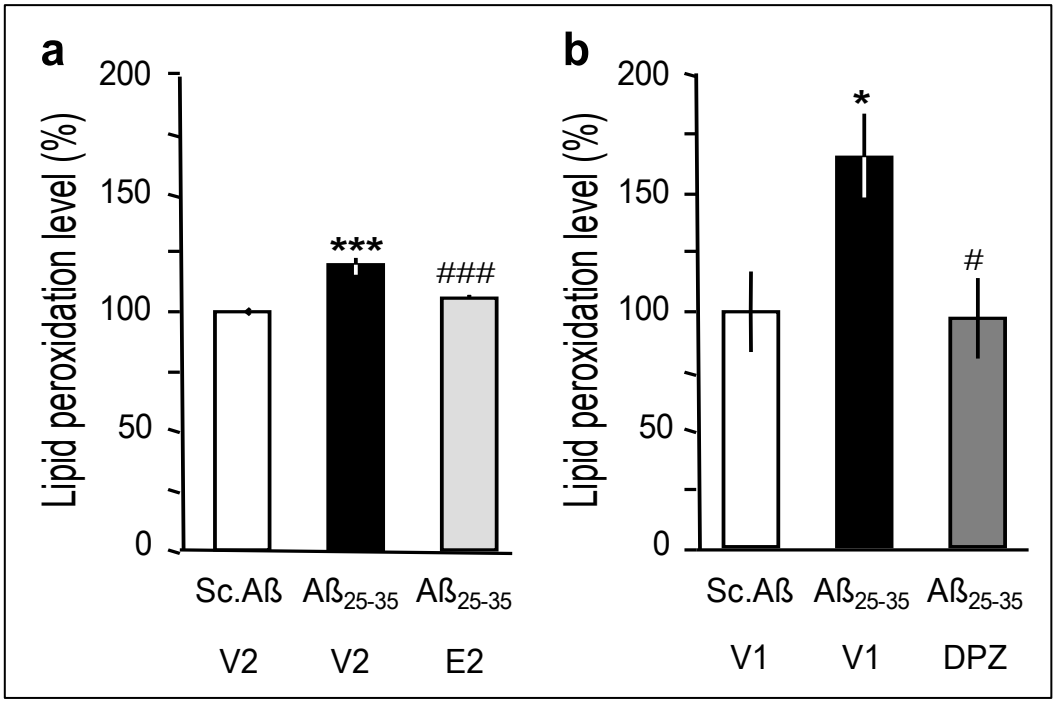

Figure 3. Protective effects of the E2 extract and donepezil on Aβ$_{25-35}$-induced lipid peroxidation in the mouse hippocampus. Abbreviations: Sc.Aβ, scrambled peptide; V1, vehicle solution 1 (distilled water); V2, vehicle solution 2 (distilled water/DMSO 90/10); DPZ, donepezil; E2, chloroform selective *Erythrina senegalensis* extract. ANOVA: $F_{(2,15)} = 19.1$, $p < 0.0001$, n = 4-8 per group in (a); $F_{(2,17)} = 5.10$, $p < 0.0001$, n = 6 per group in (b). * P < 0.05, *** P < 0.01 vs. (Sc.Aß/V) group; # P < 0.05, ### P < 0.001 vs. (Aβ$_{25-35}$/V) group; Student's *t*-test.

The acetylcholinesterase inhibitor (Donepezil) is a reference drug that was used in a separate set of experiments in order to compare the efficacy of the plant extracts as neuroprotectant in a mouse model of Alzheimer's disease. Amyloid toxicity was induced in the model by an acute ICV injection of Aβ$_{25-35}$ peptide, which has been oligomerized by incubation. The peptide was therefore in the form of short amyloid fibrils (Maurice et al., 1996; Zussy et al., 2011,2013). The toxic form of amyloid aggregates diffused throughout the choroid plexus and vascularity throughout the parenchyma in brain structures such as the cortex, the hippocampus, hypothalamus, putamen caudate, and limbic structures (Zussy et al., 2011). Toxicity developed within one week and mice exhibited learning and memory deficits, neuroinflammation, oxidative stress, cellular stress and apoptosis, and progressive accumulation of amyloid Aβ proteins and hyperphosphorylated Tau proteins (Chavant et al., 2010; Villard et al., 2011; Lahmy et al., 2013). The model is therefore highly relevant to provide a rapid *in vivo* screening approach to test potential neuroprotectors in Alzheimer's disease. Indeed, two recent studies made it possible to establish a direct comparison for the performances of a candidate drug in the acute Aβ$_{25-35}$ mouse model and in a transgenic mouse line expressing the human APP protein carrying selected mutations, for an asialic form of erythropoietin (Maurice et al., 2013; Rodriguez Cruz et al., 2017) and the acamprosate+baclofen mix (Chumakov et al., 2015). Our *in vivo* analyses therefore not only confirmed that the E2 chloroform selective *Erythrina senegalensis* extract showed a neuroprotective effect in the Aβ$_{25-35}$ mouse model of Alzheimer's disease but also that promising result may be expected from a long-term chronic treatment in a more progressive model such as transgenic mouse lines.

Study of the Chemical Composition of the Chloroform Extract

The sub-fraction D2 collected was subjected beforehand to MS analysis in order to determine its molar mass in order to facilitate its identification. Then several NMR spectra such as ^{1}H, 13C, COSY, HSQC, and HBMC have been made have been realized.

HPLC-UV Analysis of Fraction D2

To find the chemical composition of sub-fraction D2, it was first analyzed by HPLC-UV (Shimadzu Varian, 400MHz). Its chromatogram (Figure 4) revealed the mixture of 3 phytocompounds in the fraction at 3 different retention times. At 2.82 min, a minority compound appears, at 12.34 min and 13.02 min 2 major compounds are identified. This information was used in subsequent analyses during identification.

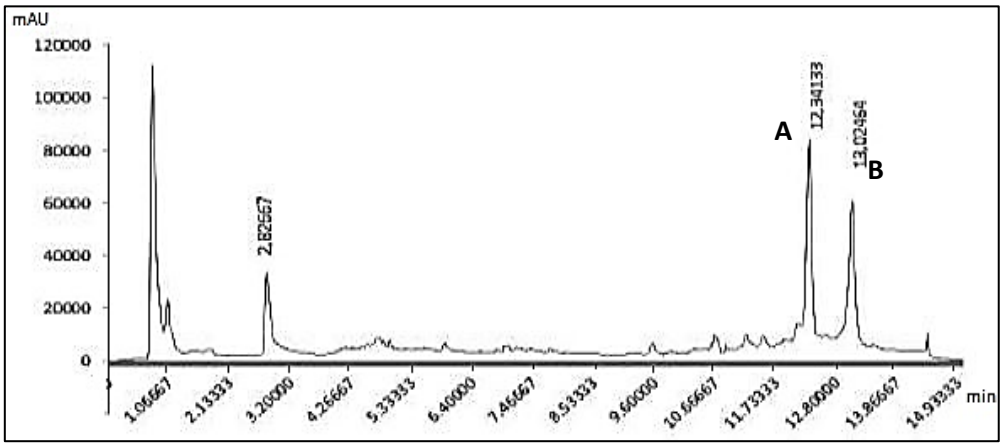

Figure 4. HPLC-UV chromatogram of fraction D2

Spectral Analysis of sub-fraction D2

The ^{1}H NMR spectrum (Figure 5) showed 2 clusters of signals, one belonging to the major compound A, and the other belonging to the minor compound B. Presumably, the interpretation of the spectrum indicates a mixture of 2 molecular structures which would belong to two subclasses of phytocompounds, namely isoflavones and flavanones (Morel et al., 2011).

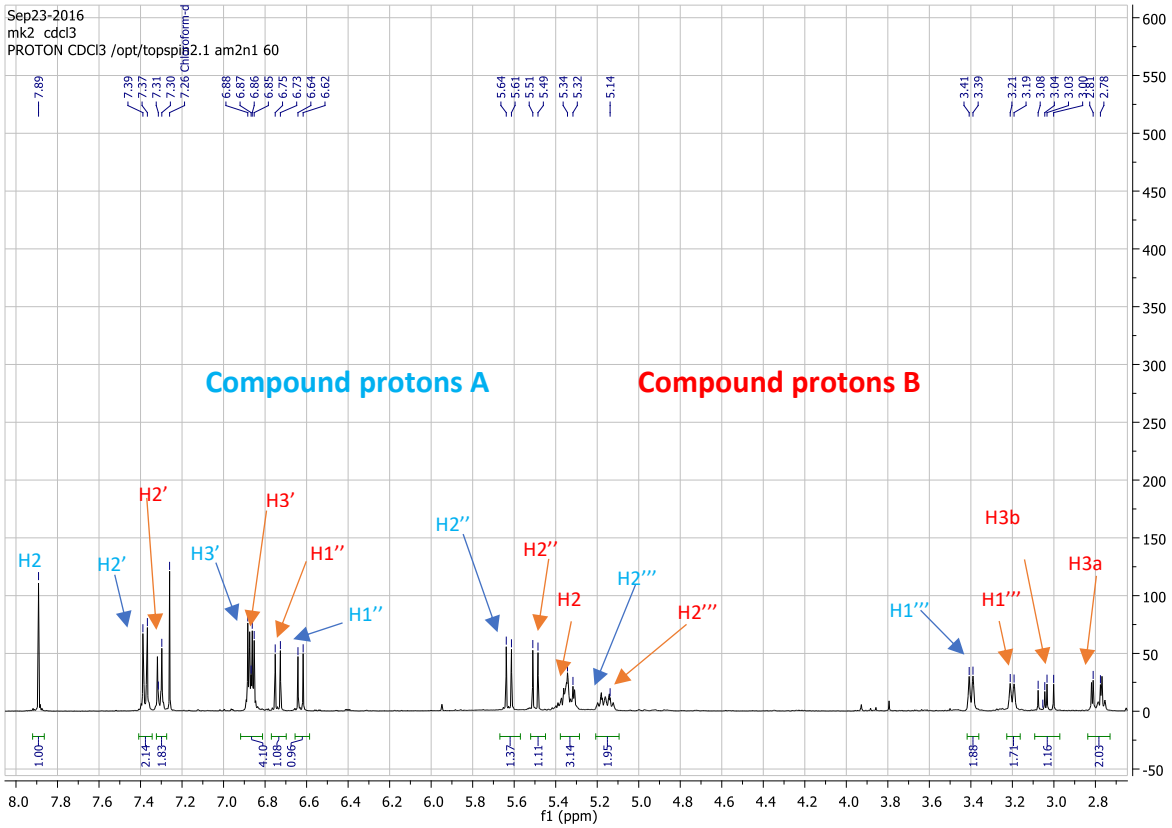

Figure 5. ^{1}H NMR spectrum of fraction D2 (400 MHz, CDCl3, TMS=0 ppm)

In addition, most of the compounds are prenylated with essentially linear prenylations of the 3,3-dimethylallyl (3,3-DMA) type and cyclic prenylations of the "pyran" type. The set of 1D and 2D, ^{1}H, ^{13}C, spin echo (number of protons bound to carbon), COSY (^{1}H-^{1}H correlation) (Figure 6), and HSQC (^{1}H-^{13}C, ^{1}J, correlation) and HMBC NMR sequences (^{1}H-^{13}C, ^{2}J, ^{3}J and ^{4}J, correlation) (Figure 8) was implemented in order to determine the structure of these two compounds and to propose an assignment of the set of chemical shifts for the ^{1}H and ^{13}C spectra.

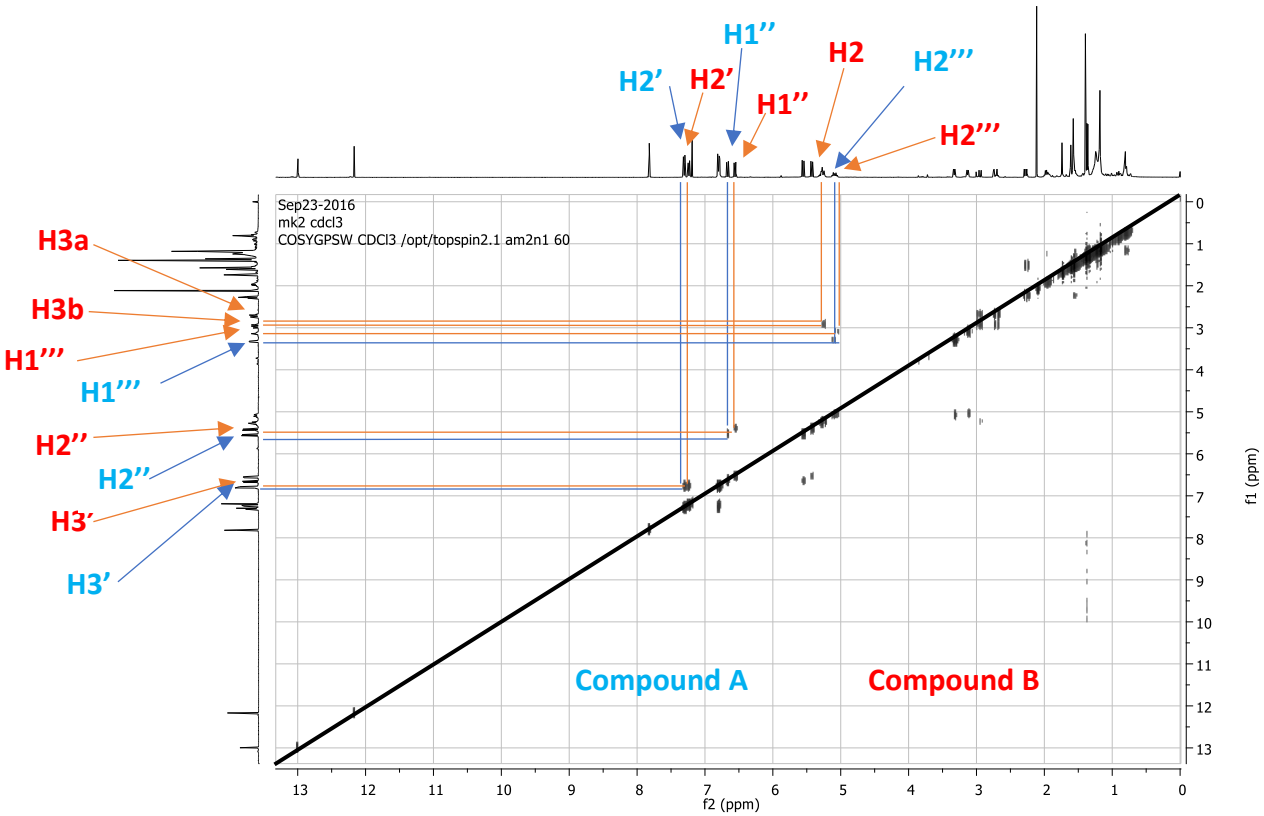

Figure 6. COSY 1H NMR spectrum of fraction D2(400 MHz, CDCl3, TMS=0 ppm)

The spin echo sequence as well as the ^{1}H (Figure 8) and ^{13}C NMR spectrum combined with the mass analysis by ESI-Q-TOF in positive mode revealed the information on the two compounds. Compound A exhibits a pseudo-molecular ion at *m/z* = 405.17 $[M+H]^{+}$, with a molar mass of 404.17uma. Likewise, compound B exhibits a pseudo-molecular ion at *m/z* = 407.18 $[M+H]^{+}$, with a molecular molar mass of 406.18uma.

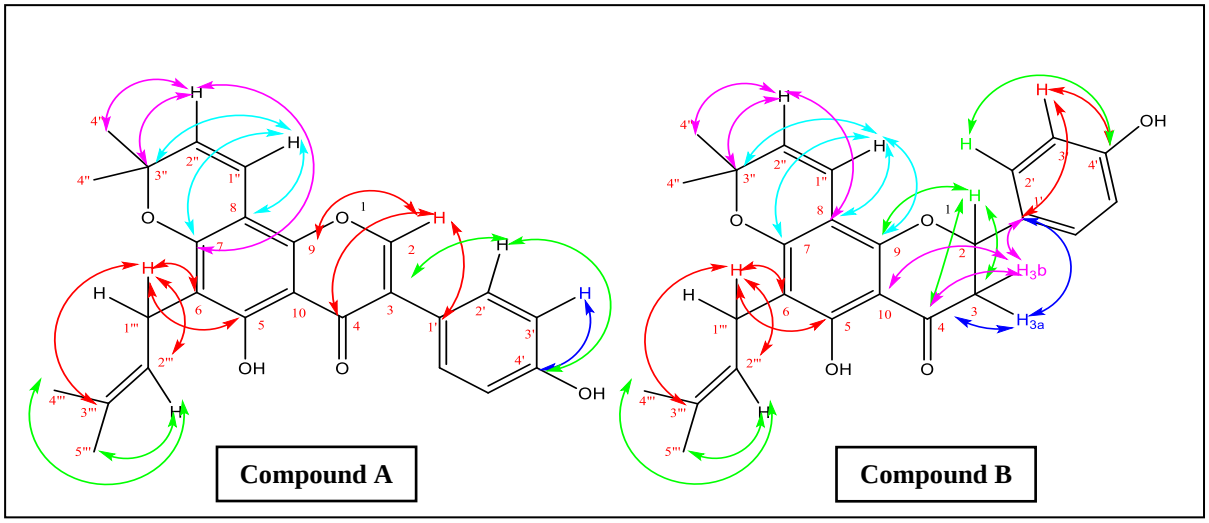

Figure 7. Molecular structure and HMBC correlation of compounds A and B

✓ ***Molecular Structure of Compound A***

Compound A has a molecular mass of 404.17uma. According to the literature, a flavonoid of the same mass, osajin was previously isolated from *Erythrina senegalensis* from Cameroon

(Donfack et al., 2008) and from *Flemingia philippinensis* (Fabaceae) from Korea (Yan Wang et al., 2013), suggesting that compound A could be this flavonoid already described which, moreover, is an isoflavone.

The ^{1}H NMR spectrum (Figure 5) is quite complex due to the large multiplicity of signals. Using the HSQC sequence, which is a technique for detecting protons directly linked to associated carbons, it is possible to make several observations; a proton (H+) resonating at 7.89 ppm in the form of a singlet is carried by C_2 at 152.76 ppm. The deshielding of this proton is due to its proximity to O1. Then two protons (2 H), resonating at 7.38 ppm in the form of a doublet are carried by $C_{2'}$ at 128.19 ppm and two other protons (2 H) resonating at 6.87 ppm are carried by $C_{3'}$ at 115.67 ppm. The high signals from these protons are a reminder of the aromatic B ring of isoflavones substituted at the 4' position. In addition, two protons (2 H) resonating in the form of two doublets (δH= 5.63-6.74ppm) couple between them with a coupling constant J = 10 Hz are carried, respectively, by the $C_{1''}$ to 115.81 ppm and $C_{2''}$ at 128.19 ppm and are characteristic of an olefinic double bond of Z geometry. The COSY ^{1}H spectrum (Figure 6) also shows their proximity.

A signal appearing as a doublet of doublets (dd) integrating for two protons (2H), (δH = 3.4 ppm), which corresponds to CH_2- of the prenylation, and which is carried by the $C_{1'''}$ to about 21.6 ppm. There is also one (1) integrating triplet for a proton (1H) at approximately 5.11 ppm corresponding to the ethylenic proton (H) carried by the $C_{1''''}$ at 122.13 ppm.

The HMBC sequence (^{1}H-^{13}C, ^{2}J, ^{3}J, and ^{4}J correlation) also made it possible from the proton-carbon correlations to draw the following conclusions:

- The H_2 proton correlating with C_3, C_4, C_9, $C_{1'}$ allows the deduction of the position of the B ring and the chemical shift δc_4= 181.52 ppm. In addition to the protons $H_{1''}$ and $H_{2''}$ correlating with C_7, C_8, $C_{3''}$ and C_7, $C_{3''}$, $C_{4''}$, respectively, lead to infer the position of the pyranic cycle.
- The $H_{1''''}$ protons which correlate with C_6, $C_{2''''}$ and $C_{5''''}$ are used to infer the 3,3-DMA position.

These three observations confirm that the backbone of this molecule is indeed a prenylated isoflavone (Table 2). These correlations make it possible to position the prenylations and to identify this compound as osajin, as described by Morel et al. (2011). On the other hand, on the ^{13}C NMR spectrum produced in C_3D_6O by Delle et al. (1994), the chemical shifts of C_2 (154 ppm), C7 (157.7 ppm), $C_{2'}$ (131.1 ppm) are different; just like in ^{1}H NMR where the chemical shifts of H_2, $H_{2'}$, $H_{3'}$ are also different from ours.

Molecular Structure of Compound B

The mass spectrum, produced in ESI-Q-TOF in positive mode, shows a pseudo-molecular peak m/z = 407.18 $[M+H]^+$, which corresponds to the crude formula $C_{25}H_{26}O_6$, which makes it possible to deduce 13 centers of unsaturations. On the one hand, several centers could correspond to the skeleton of flavonoids and, on the other hand, to the unsaturations matching to prenylations.

Analysis of the NMR ^{1}H spectrum, carried out in $CDCl_3$ (Figure 5) indicates the presence of 3 protons: H_2 (dd, δH 5.31ppm; J = 12.7 and 3.2 Hz) and 2 prochiral H_3 (dd, δH 2. 81ppm; J = 3.2 and 17.3 Hz) and (dd, δH 3.04 ppm; J = 12.7 and 17.3 Hz) corresponding to a flavanone. The HSQC spectrum made it possible to verify that the two protons of C_3 are not chemically equivalent. They are homomorphic and therefore enantiotropic.

Two doublets at 6.63 ppm (1 H), and 5.50 ppm (1 H), coupled together with a coupling constant J = 10 Hz, as well as two CH_3- groups appearing isochronous (δH at 1.4 ppm, 6H) indicate the presence of a pyran-type prenylation. The rest of the signals show the presence of a prenylation of the 3,3-DMA type: H (t, δH 5.11 ppm; J = 7.4 Hz) and (d, δH 3.20 ppm; J = 7.4 Hz) and 1.57 ppm (6H). Finally, in the aromatic H region, two doublets at 6.87 and 7.31 ppm each integrating for 2H and coupled together (J = 8.3 Hz) correspond to an AA'XX' system which can belong to the cycle B of flavanone. The presence of a resonant H in the form of a singlet at 12.24 ppm characteristic of a chelated OH group (5-OH) was also noted. The HMBC spectrum (Figure 8) shows that the H at 3.20 ppm of linear prenylation of the 3,3-DMA type correlates with C_7, while H at 6.63 ppm correlates with C_9 (Figure 5). These results were also confirmed by Morel et al. (2011).

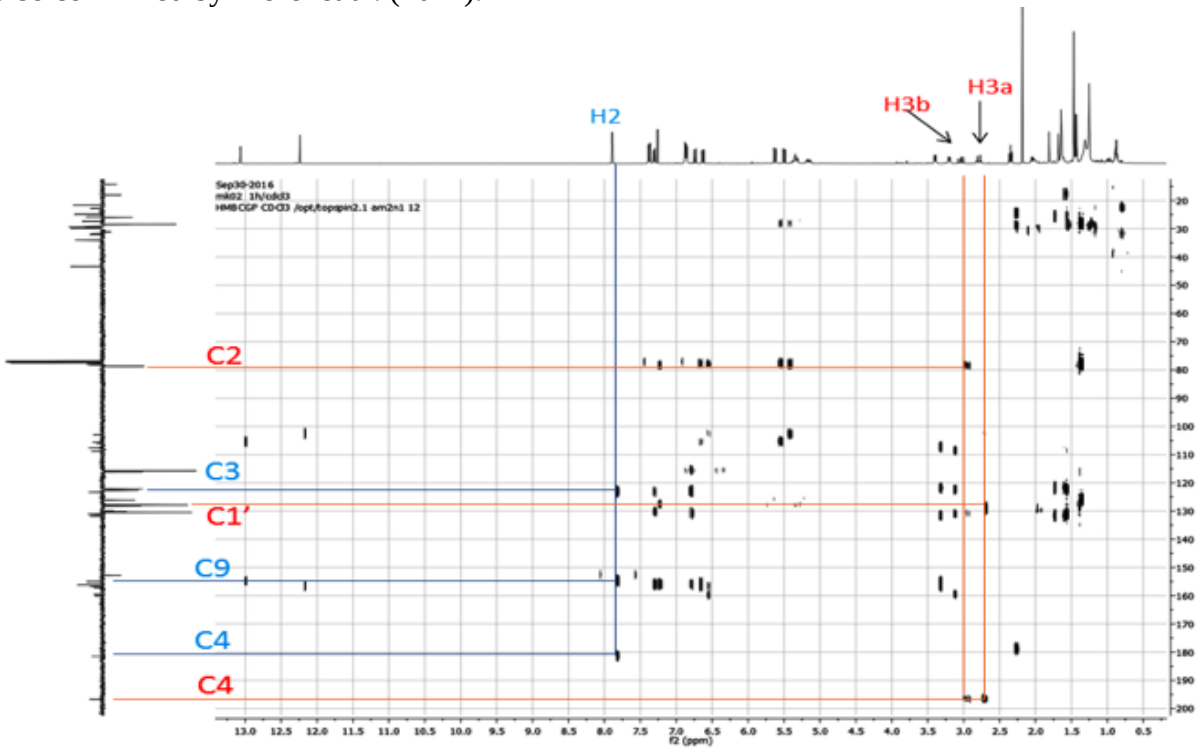

Figure 8. HMBC spectrum of fraction D2(400 MHz, CDCl3, TMS=0 ppm)

These correlations (Table 3) make it possible to position the prenylations and to identify this compound as cajaflavanone (figure 7) and which corresponds to the levorotatory (-) 2*S* enantiomer; first isolated from *Cajanus cajan* (Fabaceae), the species from India (Bhanumati et al., 1978; Morel et al., 2011).

Table 2. Spectral data of compound A

Position	**δ_C(ppm)**	**^{13}C (APT)**	**δ_H(ppm)**	**Multiplicity (*J*,Hz)**	**HMBC (**400MHz, $CDCl_3$)
1	-				
2	152.76	CH	7.89	s; 1H	3; 4; 9 ; 1’
3	123.39	C			
4	181.48	C			
5	159.52	C-OH	13.07		
6	105.62	C			
7	159.52	C			
8	107.64	C			
9	157.10	C			
10	102.82	C			
1'	130.18	C			
2'	128.19	CH	7.38	d; J=8.3Hz, 1H	3; 1'; 4'
3'	115.67	CH	6.87	d; J=8.3Hz, 1H	3; 1'; 4'
4'	156.14	C			
1"	115.81	CH	6.74	d; *J*=10Hz, 1H	7;8;3"
2"	128.19	CH	5.63	d; *J*=10Hz, 1H	8; 3"; 4"
3"	77.98	C			
4"	28.35	CH3	1.40	s; 6H	
1'"	21.44	CH2	3.32	d ; *J*=7.4 Hz ; 2H	6; 2"; 5"’
2'"	122.13	CH	5.11	(t ; *J*=7.4) ; 1H	
3'"	130.37	C			
4'"	18.03	CH3	1.74	s; 3H	
5'"	25.90	CH3	1.61	s; 3H	

Table 3. Spectral data of compound B

Position	δ_C (ppm)	^{13}C (APT)	δH (ppm)	Multiplicity (J,Hz)	HMBC (400MHz, $CDCl_3$)
1	-				
2	78.68	CH	5.31	dd ; *J*=12.7/3.2 Hz	3 ;4 ;9
3	43.35	CH_2	2.81	dd ; *J*=3.2/17.3 Hz	4; 1'
			3.04	dd ; J=12.7/17.3 Hz	4;10;1'
4	196.65	C			
5	160.03	C-OH	12.17		
6	106.07	C			
7	159.62	C			
8	108.8	C			
9	157.10	C			
10	102.96	C			
1'	130.37	C			
2'	128.22	CH	7.31	d; J=8.3Hz, 1H	3 ; 2; 4'
3'	115.75	CH	6.87	d; J=8.3Hz, 1H	3 ; 1'; 4'
4'	156.14	C			
1"	116.01	CH	6.63	d; J=10Hz, 1H	7;8;9 ; 3''
2"	128.22	CH	5.54	d; J=10Hz, 1H	8; 3"; 4"
3"	77.28	C			
4"	28.44	CH_3	1.37	s; 6H	
1'"	21.62	CH_2	3.14	d ; J=7.4 Hz ; 2H	5,6; 2'"; 5'"
2'"	122.66	CH	5.07	(t ; *J*=7.4) ; 1H	
3'"	130.48	C			
4'"	18.03	CH_3	1.74	s; 3H	
5'"	25.94	CH_3	1.57	s; 3H	

CONCLUSION

The present work studied the neuroprotective activity *in vivo* and characterized two constituents isolated from the chloroform extract of the *Erythrina senegalensis*. The study of its chemical composition made it possible to determine the structures by HPLC-UV and 1D and 2D NMR, of two flavonoids, namely osajin (isoflavone) and cajaflavanone, isolated for the first time from the Ivorian species. In addition, the neuroprotective activity of the said extract, evaluated *in vivo* on a mouse model of Alzheimer's disease, induced by the direct administration of amyloid peptide $A\beta_{25-35}$ to mice, showed beneficial effects on induced amnesia, measured by behaviors of spontaneous alternation and passive avoidance, and on the appearance of oxidative stress in the hippocampus.

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