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Structural characterization and *in vitro* biological exploration of phytoconstituents isolated from a chloroform extract of *Rauvolfia vomitoria* (Apocynaceae) root bark from Côte d'Ivoire

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Abstract
The species of the genus Rauvolfia are a reservoir of indole alkaloids with proven pharmacological properties. A chloroform extract of the root bark of *Rauvolfia vomitoria* (Apocynaceae) was studied. The phytochemical investigation of said extract allowed the isolated of four indolic alkaloids, three of which are known (Reserpine, ajmaline and mauiensine) and the unknown phyto-constituent (3). In addition, the methyl esters of 3, 4, 5-trimethoxybenzoic acid (Phytoconstituent (1)) and 3, 4, 5-trimethoxycinnamic acid (Phytoconstituent (2)) were characterized and considered as new products of reserpine degradation. Their molecular structures were determined by MS, NMR (^{1}H, ^{13}C), IR, UV and their melting points were established. The evaluation of the antioxidant activity *in vitro* revealed the antioxidant efficiency expressed by the DPPH radical reduction concentration at 50% (CR_{50}) of the phytoconstituents (3) (CR_{50}= 0.346 mg/mL) and (5) (CR_{50}= 0.127 mg/mL) compared with vitamin C (CR_{50} = 0.003 mg/mL). The *in vitro* study of the anticholinesterase activity towards galantamine, showed a clear anticholinesterase activity with percentages of acetylcholinesterase inhibition higher than 50% of the phytoconstituents (4) and (5). Moreover, phyto-constituent (5) showed the best antioxidant and anticholinesterase activity. These results presage promising research, including toxicological evaluation for therapeutic phyto-formulations.

Keywords: *Rauvolfia vomitoria*, phytoconstituents, structural determination, antioxidant, anticholinesterase

1. Introduction
Rauvolfia vomitoria belonging to the botanical family Apocynaceae, is a plant native to tropical Africa. The leaves, root bark and stems of the plant are used in decoction, infusion or maceration in traditional medicine to treat malaria, epilepsy, jaundice, high blood pressure, mental disorders [1-3]. The plant organs (Leaves, roots, stem) contain monoterpene indole alkaloids (MIA) ajmaline, yohimbine, reserpine, ajmalicin, serpentine type sarpagine and suaveoline type MIA [4, 5]. These secondary metabolites of the plant seem to govern its various pharmacological activities [4, 5]. Alkaloids are secondary organic nitrogenous bases for the most part heterocyclic of plant origin, and endowed with multiple biological, therapeutic and pharmacological virtues.
In this work, the choice of the plant was not made fortuitously. Indeed, it was motivated by the fact that to date, no study reports scientific investigations carried out on the extracts of total alkaloids of the root bark of the species *R. vomitoria* growing in the spontaneous state in Côte d'Ivoire. The study has a double objective. Firstly, to explore the root bark of the species from the Ivorian floristic biodiversity as a potential source of alkaloids, and secondly, to estimate the antioxidant and anticholinesterase abilities of phytoconstituents isolated for the first time from this plant matrix.

2. Material and Methods
2.1 Plant material
R. vomitoria root bark was collected from Adiopodoumé (latitude .5.34145° or 5° 20' 29" north; longitude -4.13323° or 4° 7' 60" west) in the autonomous district of Abidjan (5° 20′ 11″ north, 4° 01′ 36″ west) in southern Côte d'Ivoire. The plant was identified and authenticated by botanists at the Centre National Floristique (CNF) in Abidjan under No. 11676.

After cleaning and drying at room temperature in a ventilated room for 15 days, the plant material was milled using an electric grinder, and the powders were stored in hermetically closed glass jars.

2.2 Biological material
Acetylcholinesterase (AChE) from Electrophorus electricus (C3389-2KU), acetylthiocholine iodide (ATCI), 5, 5′-dithiobis-(2-nitrobenzoic acid) (DTNB), Galantamine hydrobromide (C17H21NO3. HBr; 368.27 g/mol), stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) and ascorbic acid (vitamin C) were purchased from Sigma Aldrich (Poole, UK).

2.3 Selective extraction of total alkaloids
320 g of grindings are macerated in 1700 mL of methanol (MeOH) for 72 h under continuous stirring. The operation is repeated 3 times. The filtrates resulting from the vacuum filtration were concentrated under reduced pressure (218 mbar) at 40 °C, then treated with 150 mL sulfuric acid (H_2SO_4, 2%, v/v, pH= 2). The acidic solution was delipidated with 4 × 100 mL of diethyl ether (Et_2O), then basified with sodium hydroxide solution (NaOH, 25%, pH= 9), and successively exhausted with 5 × 100 mL of chloroform ($CHCl_3$), ethyl acetate (AcOEt), and the AcOEt/MeOH mixture. The resulting organic fractions were dried over anhydrous sodium sulfate (Na_2SO_4), filtered, and then concentrated to dryness on the Büchi Rotavapor R-300 rotary evaporator at 40 °C. Dry extracts with chloroform, ethyl acetate and MeOH/AcOEt mixture (3/1) were obtained [6, 7].

2.4 Fractionation and isolation of phytoconstituents
1g of chloroform extract is introduced into a column containing 50 g of 60 GF254 silica gel (Merck) (length 45 cm, diameter 4 cm, silica height 17 cm) is eluted with a CH_2Cl_2 (dichloromethane)/MeOH solvent gradient (from 25: 1 to 4: 1) to obtain 5 fractions (F1-F5) combined with respect to similar TLC profiles (60 F254, aluminum support, Merck). F1 (84.8 mg) is fractionated on column chromatography (CC) to give several sub-fractions (F1.1 - F1.4). Thus, phytoconstituents (1) (1.9 mg) and (2) (6.3 mg) were isolated from F1.1 by preparative plate chromatography (PP) with a CH_2Cl_2/MeOH (10: 0.7) solvent gradient. Phytoconstituent (3) (1.4 mg) was obtained by PP with CH_2Cl_2/MeOH (10: 0.5) from F1.2. Phytoconstituent (4) (15.6 mg) was obtained from F1.3 using DCM/MeOH (100: 0 to 100: 1). F4 (490 mg) was fractionated on CC with a CH_2Cl_2 /EtOH (97: 3 to 75: 25) solvent gradient to provide 4 sub fractions (F4.1- F4.4). Phytoconstituent (5) (142.6 mg) is obtained an additional separation from F4.2 (242.39 mg) on CC by elution with CH_2Cl_2/MeOH. Similarly, phytoconstituent (6) (5 mg) is obtained from F4.4 (135.5 mg) by elution with toluene/ AcOEt/EtOH (ethanol).

2.5 Structural characterization of isolated phytoconstituents
The structures of the isolated phytoconstituents are elucidated from spectral data (HPLC, HRMS, ^1H, ^{13}C, IR, UV NMR). The 1D and 2D NMR spectra are recorded on a Bruker AVANCE III 400 spectrometer (^1H: 400 MHz, ^{13}C: 101 MHz). HPLC is performed on HPLC MS 1260_InfinityII with a C18-AGILENT column in reverse phase at 30 °C. HRMS spectra were obtained on Bruker maXis mass spectrometer in negative (ESI-) or positive (ESI+) mode (ESI) by the "Fédération de Recherche" ICOA/CBM platform (FR2708). MS analyses were performed using a Q-TOF Impact HD mass spectrometer equipped with the electrospray ion source (ESI) (Bruker Daltonics). UV-vis and IR spectra were obtained respectively on UV-vis spectrophotometer (ULTRAVIOLET SPECTRUM JASCO V-630 1036) in MeOH and IR spectrometer (380 FT Nicolet from Thermo Fisher scientific) in CH_2Cl_2. Column chromatography (CC) was performed on silica gel 60 GF254 (MERCK).

2.6 Assay of antioxidant activity by the DPPH test of isolated phytoconstituents
Evaluation of antioxidant activity of phytoconstituents (1-5) was performed according to the Blois method [8]. DPPH was solubilized in MeOH to obtain a 0.03 mg/mL solution. Different concentration ranges (0.5; 0.25; 0.125; 0.0625; 0.03125; 0.0156; 0.0078 mg/mL) of each plant extract are prepared in the same solvent. To test tubes containing 1 mL of each methanolic solution of plant extract obtained at the different concentrations, 1.5 mL of DPPH solution is added. After shaking, the tubes are placed in the dark for 30 min, and then the absorbance of the reaction mixture is measured at 517 nm with a UV-vis spectrophotometer against a blank (1.5 mL of DPPH solution in 1 mL of MeOH. The reference antioxidant is vitamin C, prepared under the same conditions as the tested samples. The percentage reduction (PR) of DPPH is calculated according to the formula:

$$PR\ (\%) = (A_b - A_e) / A_b \times 100$$

%PR: reduction percentage; Ab: absorbance of the blank; Ae: absorbance of the sample.
The median DPPH reduction concentration (CR_{50}) which reflects the antioxidant efficiency of the extract is determined graphically [9, 10].

2.7 Determination of anticholinesterase activity of isolated phytoconstituents
AChE inhibitory activity was performed according to the slightly modified method of Ellman [11]. In 96-well plates, 50 µL of the samples (500; 250; 125; 62.5; 31.25; 15.625; 7.81 µg/mL in MeOH) were diluted 1/4 with the buffer solution to obtain final readout concentrations and 10 µL of the AChE enzyme (0.22 U/mL in Tris-HCl buffer) incubated for 30 min at 37°C. Then 20 µL of DTNB (3 mM in buffer) and 10 µL of ATCI (15 mM, H_2O millipore) are added, and the plates were placed in the dark for 5 min at 18°C. Absorbance is measured at 405 nm every 90 s for 6 min three times consecutively. A control (galantamine) and a blank (MeOH) were prepared under the same conditions. The percentage inhibition (PI) of the enzyme is calculated according to the following formula:

$$PI\ (\%) = 100 - [(A_e / A_b) \times 100]$$

PI = percentage inhibition of AchE; Ab: absorbance of the blank; Ae: absorbance of the sample; Buffer: 50 mM Tris-HCl; pH= 8.

3. Results and discussion
3.1 Extraction yields
Table 1 shows the yields of selective extraction of total alkaloids obtained by successive exhaustion using solvents of varying polarity. The best extraction yield is obtained with $CHCl_3$.

Table 1: Yield of selective extraction of total alkaloids from ground material

Extraction solvent	Ground
	Yield (%)
$CHCl_3$	7
AcOEt	1, 14
MeOH/AcOEt	5, 9

3.2 Structural profiles of isolated phytoconstituents: Figure 1 shows the molecular structures of the phytoconstituents isolated from the chloroform extract of the root bark of *R. vomitoria*.

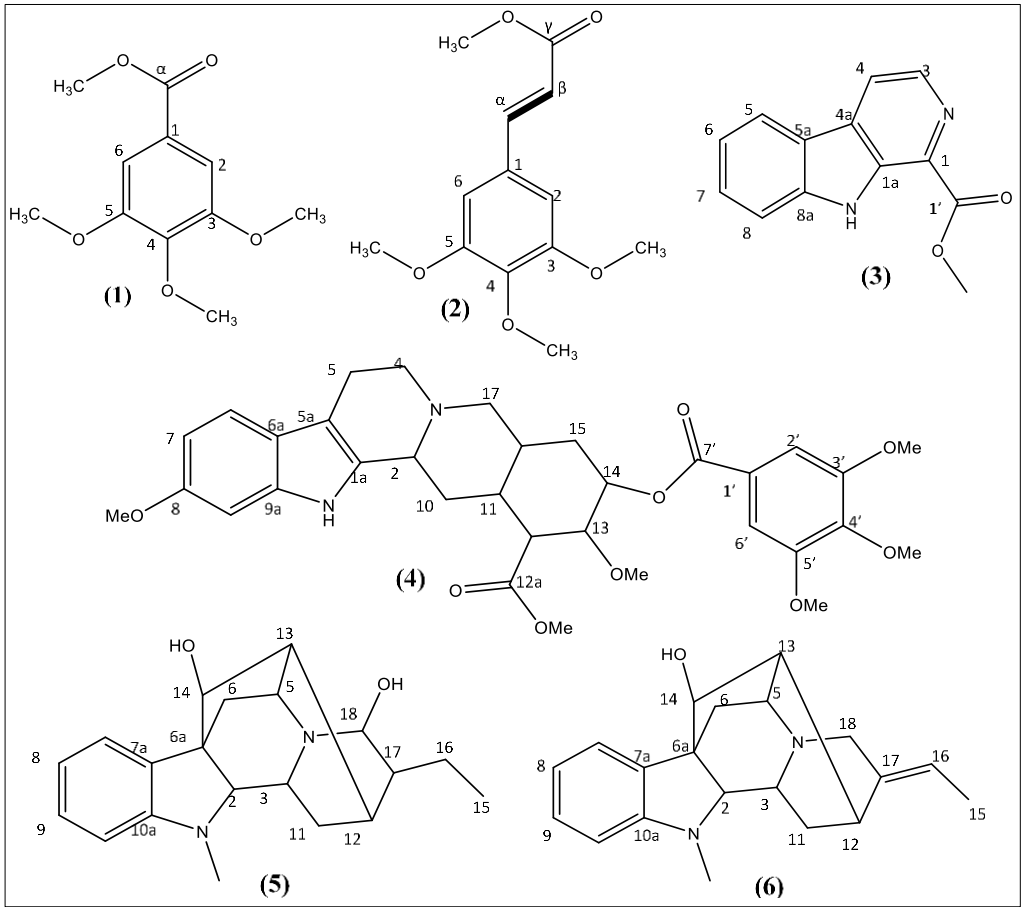

Fig 1: Isolated phytoconstituents from chloroform extract

The phytoconstituent (1) Methyl 3, 4, 5-trimethoxybenzoate (Figure 1) is a white amorphous solid (Rf= 0.35; Petroleum Ether (PE)/AcOEt, 8: 2), Tf= 83 °C, retention time (Tr= 10 min) on HPLC showed molecular ion peaks on HRMS at m/z 227.09 $[M+H]^+$ and 249.07 $[M+Na]^+$, referring to the gross formula $C_{11}H_{14}O_5$. Its fragmentation gives son ions at m/z 195 $[M+H-CH_4O]^+$; 183 $[M+H-CO_2]^+$ and 168 $[M+H-CO_2CH_3]^+$. The IR spectrum shows an absorption band at 1157 cm^{-1} (C-O); 1722 cm^{-1} (C=O). The UV spectrum shows absorption maxima (ʎ max: nm (log Ɛ) at 202 (268.63); 211 (204.20) and 264 (53.85). The ^{13}C NMR spectrum (Table 2) shows 11 signals corresponding to 11C: Cα (166.88 ppm); C3 or C5 (153.08 ppm); C4 (144.10 ppm); C1 (125.29 ppm); C2 or C6 (106.93 ppm); C4' (61.07 ppm); C3' or C5' (56.38 ppm) and Cα' (52.38 ppm). The ^1H NMR spectrum indicates the presence of 2 aromatic H in the ortho position at 7.3 ppm (H2 or H6), 6 H at 3.90 ppm (C3-OCH_3 and C5-OCH_3) and 3 H at 3.87 ppm (C4-OCH_3) and 3 H at 3.90 ppm (Cα-OCH_3) corresponding to -OCH_3 groups. The ^1H-^{13}C HSQC and HMBC correlations (Figure 2) are presented in Table 2.

The phytoconstituent (2) Methyl (E)-3-(3, 4, 5-trimethoxyphenyl) acrylate (Figure 1) is a white amorphous solid (Tf= 99.93 °C; Rf= 0.28 (EP/AcOEt 8/2); Tr = 12.74 min by HPLC. Its ESI-Q-TOF-MS mass spectrum shows peaks at m/z 253.11 $[M+H]^+$ and 221.08 $[M+H-CH_4O]^+$ which combined with ^{13}C NMR data, established its molecular formula $C_{13}H_{16}O_5$. Its ^{13}C NMR spectrum indicates that the molecule has 13 C including 2 C methine (-CH-) more than the phytoconstituent (1) for which the signals appear at 145.00 ppm (Cα) and 117.18 (Cβ). As for the 11 C, their chemical shifts are similar to those of phytoconstituent (1) (Table 2). In addition, the ^1H NMR spectrum of phytoconstituent (2) is identical to that of phytoconstituent (1) except for the presence of olefinic 2 H signals of the double bond of geometry (E) at 7.61 ppm (1H, d, J = 15.9 Hz, Hα) and 6.35 ppm (1H, d, J = 15.9 Hz, Hβ). Correlation of the COSY 1H-1H spectrum shows a relationship between Hα (7.61 ppm) and Hβ (6.35 ppm), which is confirmed by the HSQC and HMBC spectra. The 2 H are carried by adjacent carbons in resonance with Cα' (145 ppm) and Cβ' (117.18 ppm) (Table 2). The IR spectrum of phytoconstituent (2) is similar to that of phytoconstituent (1) except that in the former, the absorption band of H_3C-OC=O at 1709 cm^{-1} is slightly different, since it is conjugated with the C=C double bond. The absorption band at 3148 cm^{-1} corresponds to olefinic CH.

The comparison of the spectral data set obtained for the phytoconstituents (1, 2) and the analytical data obtained corroborate with the information described in the literature [12, 13].

This allowed defining the phytocompounds as Methyl 3, 4, 5-trimethoxybenzoate (1) and Methyl(E)-3-(3, 4, 5-trimethoxyphenyl) acrylate (2) (Figure 2).

Table 2: ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) and HMBC of compound (1, 2)

N°	Type	^{13}C δ (ppm)		H (m, J Hz)		HMBC	
		(1)	(2)	(1)	(2)	(1)	(2)
1	C	125.29	130.02				
2 = 6	CH	106.93	105.34	7.3 (1H, s)	6.75 (1H, s)	1,2,3,4,5,6; α(1)	1; 2; 3; 4; 5; 6; α(2)
3 = 5	C	153.08	153.58				
4	C	144.10	140				
α(1)	C=O	153.08					
α (2)	CH		145.00		7.61 (1H, d, *J* = 15.9 Hz)		1; 2; β(2); γ(2)
β(2)	CH		117.18		6.35 (1H, d, *J* = 15.9 Hz)		1; γ(2)
γ(2)	C=O		167.54				
3'= 5'	3-OCH_3 ou 5-OCH_3	56.38		3.90 (6H, s)	3.89 (6H, s)	3; 5	3; 5
4'	4-OCH_3	61.07	61.12	3.87 (3H, s)	3.81 (3H, s)	4	4
α'(1)	α-OCH_3	52.38		3.91 (3H, s)		α'(1)	
γ'(2)	γ-OCH_3		51.87		3.88 (3H, s)		γ'(2)

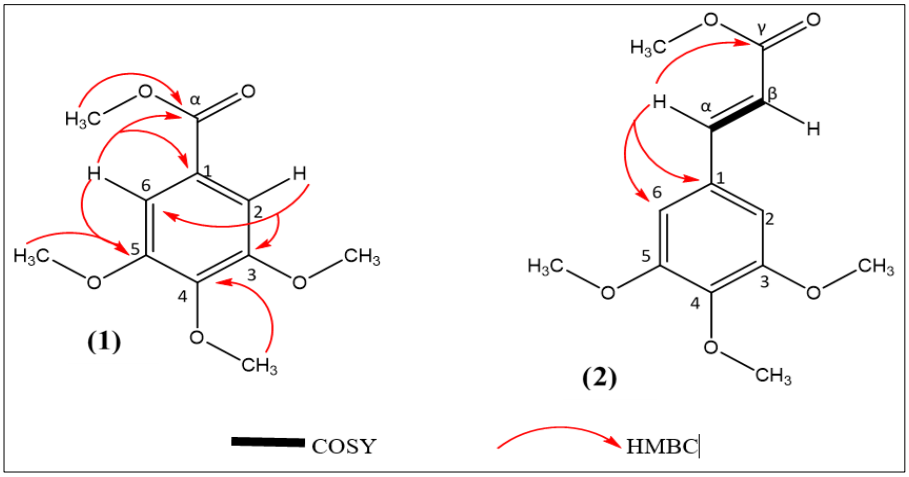

Fig 2: COSY and HMBC correlations of phytoconstituents (1, 2).

The phytoconstituent (3) Methyl 9H-pyrido [3, 4-b] indole-1-carboxylate (Figure 1) appeared as pale yellow spikes (Tf= 170°C); Rf= 0.4 (CH_2Cl_2/MeOH 10/0.5), Tr = 5.60 min in HPLC. Its positive mode ESI-Q-TOF-MS mass spectrum showed a molecular ion at m/z 227.08 $[M+H]^+$, consistent with the gross formula $C_{11}H_{10}N_2O_2$. Its fragmentation gives rise to son ions at m/z $[M+H-CH_2]^+$; 185 $[M+H-C_2H_2O]^+$ and 167 $[M+H-HCO_2CH_3]^+$. The UV-vis spectrum (ʎmax nm (log Ɛ)), MeOH: 202 (61.853); 273 (4.867); 302 (3.213); 368 (2.241) characteristic of the ß carbolinium chromophore [14]. The IR spectrum highlights N-H (3389 cm^{-1}), aromatic C-H (2918 cm^{-1}), C=O (1783 cm^{-1}), aromatic C=C (1658 cm^{-1}) and indole C=C (1410 cm^{-1}) absorption bands. The ^{1}H NMR spectrum (Table 3) of phytoconstituent (3) indicates the presence of an indole ring by the appearance of signals at 8.19 ppm (1H, d, J= 8 Hz, H5), 7.63 ppm (1H, d, J=7.7 Hz, H7), 7.60 ppm (1H, d, J = 7.7 Hz, H8), 7.35 ppm (1H, m, H6) and 9.91 ppm (1Hs, NH). Signals at 8.60 ppm (1H, d, J = 5.0 Hz, H3), 8.16 ppm (1H, d, J = 5 Hz, H4) correspond to trisubstituted pyridine protons. This spectrum also shows a signal at 4.11 ppm (3H, s, OMe) indicating the presence of a carbomethoxy group. The ^{13}C NMR spectrum also shows the characteristic signals of a substituted indole ring: 5 quaternary C C1 (156.27 ppm), C8a (155.18 ppm), C5a (123.71 ppm), C1a (131.70 ppm), C4a (122.13 ppm) and 6 tertiary C C3 (139.10 ppm), C4 (118.22 ppm), C5 (122.18 ppm), C6 (118.92 ppm), C7 (129.66 ppm), C8 (112.03 ppm). In addition, it shows the peak corresponding to the C=O carbon at 166.22 ppm, assigned to C1'. The presence of the carbomethoxy group bound to C1 is confirmed by the C(OMe) signal resonating at 52.99 ppm. The ^{1}H-^{1}H COSY spectrum gives the correlations H3 (1H, d, J=5 Hz, 8.60 ppm) / H4 (1H, d, J=5 Hz, 8.16 ppm), and H5 (1H, d, J=8 Hz, 8.19 ppm) / H6 (1H, m, 7.35 ppm); H7 (1H, d, J=7.7 Hz, 7.63 ppm) / H6 (1H, m, 7.35 ppm) / H8 (1H, d, J = 7.7 Hz, 7.60 ppm) which establishes the - (CH - CH) - (CH -CH) - chain (Figure 3). The chemical shift observed at 52.99 ppm correlates with the shift at 4.41 ppm in the HMBC spectrum (Figure 3), suggesting the presence of the OMe moiety and confirming its position associated with carbon C1. From these results, as well as a direct comparison of the above data with those published in the literature, the structure of phytoconstituent (3) was determined to be 1-carbomethoxy-β-carboline [15-17].

Table 3: ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), COSY and HMBC of compound (3)

N°	Type	^{13}C δ (ppm)	H (m, J Hz)	COSY	HMBC
1'	C=O	166.22			
1	C	156.27			
1a	C	131.70			
3	CH	139.10	8.60 (1H, d, J = 5.0)	H4	
4	CH	118.22	8.16 (1H, d, J=5)	H3	
4a	C	122.13			
5	CH	122.18	8.19 (1H, d, J= 8)	H6	
5a	C	123.71			
6	CH	118.92	7.35 (1H, m)	H5 H7	
7	CH	129.66	7.63 (1H, d, J=7.7)	H6 H8	
8	CH	112.03	7.60 (1H, d, J = 7.7)	H7	
8a	C	155.18			
	NH		9.91 (1H, s)		
	O(CH_3)	52.99	4.11 (3H, s)		1

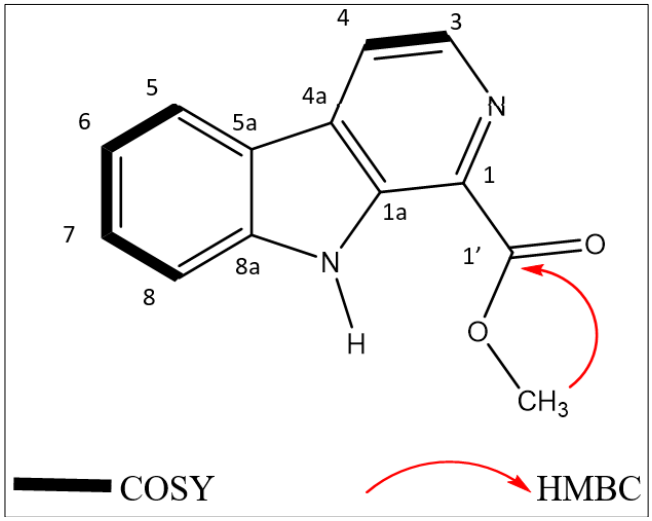

Fig 4: Correlations COSY and HMBC of phytoconstituent (3).

The phytoconstituent (4) Methyl 2,11-dimethoxy-3-((3,4,5-trimethoxybenzoyl) oxy)-1,2,3,4a,5,7,8,13,13b14,14a-dodecahydroindolo[2',3',3,4]pyrido[1,2-b]isoqinolin-1-carboxy-late) (reserpine) (Figure 1) is an off-white solid (Rf= 0.47; CH_2Cl_2/MeOH, 15: 1), Tf= 264 °C. The UV spectrum showed absorption maxima (ʎmax nm (log Ɛ)), at 217 (449.90); 268 (112.47); 305 (44.83), characteristic of the indole chromophore, and the IR spectrum showed absorption bands 3389 cm^{-1} (NH), 1709 cm^{-1} (ketocarbonyl), 1044 cm^{-1} (C-N), -C-H (2956 cm^{-1}), 1044 cm^{-1} (typical to methoxyindole) and 927-887 cm^{-1} (-C-H of the substituted benzene ring). Its mass spectrum obtained on HRMS QTOF MS ESI$^+$ showed a molecular ion at m/z 609.28 $[M+H]^+$, corresponding to the gross formula $C_{33}H_{40}N_2O_9$. The ^{1}H NMR spectrum (Table 4) showed the presence of signals from 6 CH_3O- groups as singlet at 7.32 ppm (2H, H2' and H6'), 3.91 ppm (6H, 3'-OCH_3 and 5'-OCH_3), and at 3.92 ppm (3H, 4'-OCH_3); a mass of doublets at 5.05 ppm (1H, ddd, J=9.2 Hz, J=5.1 Hz and J=11.7 Hz, H13) indicating the presence of the C14 methyl carboxylate ester. The ^{1}H and ^{13}C NMR spectra indicate that the phytoconstituent (4) contains a resonant CH_3OC=O moiety as a singlet at 3.83 ppm (3H, s, 12a-OMe), C(12a-OMe) at 52.02 ppm and C12a (172.96 ppm) and 2 CH_3O- at 3.85 ppm (3H, s, 8-OMe) and 3.51 ppm (3H, s, 13-OMe), C(8-OMe) at 55.96 ppm and C13 (77.94 ppm). A singlet belonging to 1 H at 7.54 ppm was assigned to N-H, and a doublet at 7.34 ppm (1H, d, J =3.6 Hz), a singlet at 6.85 ppm (1H), and a doublet of doublets at 6.78 ppm (1H, J =8.6 Hz, 2.2 Hz, H6) were assigned to the aromatic H6, H7, and H9, respectively, indicating that one of the CH_3O- groups is at C8. Two doublets of doublets at 5.05 ppm (J=11.7 9.2 Hz) and 2.7 ppm (J = 4.6 11.2 Hz) assigned to H13 and H12 showed the position of methoxy ester and a CH_3O- at C12 and C13, respectively. Its ^{13}C NMR spectrum, DEPT confirmed the presence of 33 C signals, including 6 methoxy-, 5 methylenes, 11 methines (5 sp2 and 6 sp3) and 11 quaternary Cs (9 sp2, and 2 C=O) (Table 4). Le spectre ^{1}H-^{1}H COSY (Tableau 4) montre les corrélations H2 (t, J = 3,0 Hz, 1H, 3,90 ppm) et H10a,b (d, J = 13,5, 4,8 Hz, 2,35 et 1,99 ppm); H4a,b (d, J = 10,5 Hz, 1H, 3,66 ppm; dd, J = 10,7, 4. 8 Hz, 1H, 3,19 ppm) / , H5a (m, 1H, 2,95 ppm; m, 1H 2,49 ppm); H6 (d, J = 3,6 Hz, 1H, 7,34 ppm) / H7 (dd, J = 8.6, 2. 2 Hz, 1H, 6,78 ppm); H11 (dd, J = 14.0, 4.2 Hz, 1H, 2,07 ppm) / H12 (dd, J = 11.2, 4.7 Hz, 1H, 2,70 ppm) / H10a,b; H13 (ddd, J = 11,7, 9,2, 5,1 Hz, 1H, 5,05 ppm) / H12 (dd, J = 11,2, 4,7 Hz, 1H, 2,70 ppm) / H14 (d, J = 5,0 Hz, 1H, 4,49 ppm); H15a,b (dd, J = 13,5, 4,8 Hz, 1H, 2,31 ppm; dd, J = 13,0, 4,4 Hz, 1H, 1,99 ppm) / H14 (d, J = 5,0 Hz, 1H, 4,49 ppm) / H16 (m, 1H, 1,92 ppm); H17a,b (dd, J = 11,7 3,6 Hz, 1H, 3,06 ppm; d, J = 5,1 Hz, 1H, 2,48 ppm) / H16 (m, 1H, 1,92 ppm). The observed correlations at 7.54 ppm (NH) / C6a (122.27 ppm), C5a (108.27 ppm); 3H (8-OMe) / C8, 3H (12a-OMe) /C12a, 3H (13-OMe) / C13, 3H (3' 5'-OMe) / C(3' 5') and 3H (4'-OMe) / C4'. The HMBC spectrum (Figure 4) defined the different positions of CH_3O- at C8, C12a, C13, C3' 5', C4' respectively. The NOESY correlations (Table 4) at 7.54 ppm (NH) / H9 (6.85 ppm) / 3H (8-OMe); 3H (13-OMe) / H6' (7.32 ppm), justify the respective location of the methoxy (8-OMe) at C8 and at the location of the methyl carboxylate ester at C14. Comparison of the above spectroscopic data with literature identified the phytoconstituent (4) being as reserpine [18].

Table 4: ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, CDCl3), COSY, HMBC and NOESY of phytoconstituent (4)

N°	Type	^{13}C δ (ppm)	H (m, J Hz)	COSY	HMBC	NOESY
1'	C	125.52				
2'=6'	CH	106.87	7.32 (s, 1H')		1'; 2'; 3'; 4'; 5'; 6'; 7'	
3'=5'	C	153.11				
4'	C	142,36				
1a	C	130.37				
2	CH	78.09	3.90 (t, J = 3.0 Hz, 1H),	H10a, b		
4	CH_2	51.27	3.66 (d, *J* = 10.5 Hz, 1H, Ha) 3.19 (dd, *J* = 10.7, 4.8 Hz, 1H, Hb)	H5a, b		
5	CH_2	16.91	3.01 – 2.90 (m, 1H, H5a) 2.54 – 2.49 (m, 1H, H5b),	H4a, b		
5a	C	108.27				
6	CH	118.74	7.34 (d, *J* = 3.6 Hz, 1H)	H7	8; 9a	H7
6a	C	122.27				
7	CH	109.23	6.78 (dd, *J* = 8.6, 2.2 Hz, 1H)	H6	6a; 9	H6
7'	C=O	165.54				
8	C	156.40				
9	CH	95.30	6.85 (s, 1H)		6a; 7; 8	8-OMe
9a	C	136.47				
10	CH_2	29.84	2.35 (d, *J* = 13.5, 4.8 Hz, 1H H10a) 1.99 (dd, J = 13.0, 4.4 Hz, 1H, H10b)	H2; H11		
11	CH	32.39	2.07 (dd, J = 14.0, 4.2 Hz, 1H)	H10a,b; H12		
12	CH	51.90	2.70 (dd, J = 11.2, 4.7 Hz, 1H)	H11; H13		

12a	C=O	172.96				
13	CH	77.94	5.05 (ddd, J = 11.7, 9.2, 5.1 Hz, 1H)	H12; H14		
14	CH	53.81	4,49 (d, J = 5.0 Hz, 1H),	H13; H15a,b		
15	CH_2	24.43	2.31 (dd, *J* = 13.5, 4.8 Hz, 1H, H15a) 1.81 (dd, *J* = 13.0, 4.4, Hz, 1H, H15b)	H16; H14		
16	CH	34.13	1.96 – 1.89 (m, 1H	H15a,b, H17a,b		
17	CH_2	49.10	3.06 (dd, J = 11.7, 3.6 Hz, 1H, H17a) 2.48 (d, J = 5.1 Hz, 1H, H17b)	H16		
	3’ 5’-OMe	56.40	3.91 (s, 6H, 3’ 5’-OMe)		3’ 5’	
	4’-OMe	61.09	3.92 (s, 3H, 4’-OMe),		4’	
	8-OMe	55.96	3.85 (s, 3H, 8-OMe) 3.85 (s, 3H, 8-OMe)		8	
	12a-OMe	52.02	3.83 (s, 3H, 12a-OMe)		12a	
	13-OMe	60.94	3.51 (s, 3H, 13-OMe)		13	H6’
	NH		7.54 ppm (s, 1H)		5a; 6a	H9

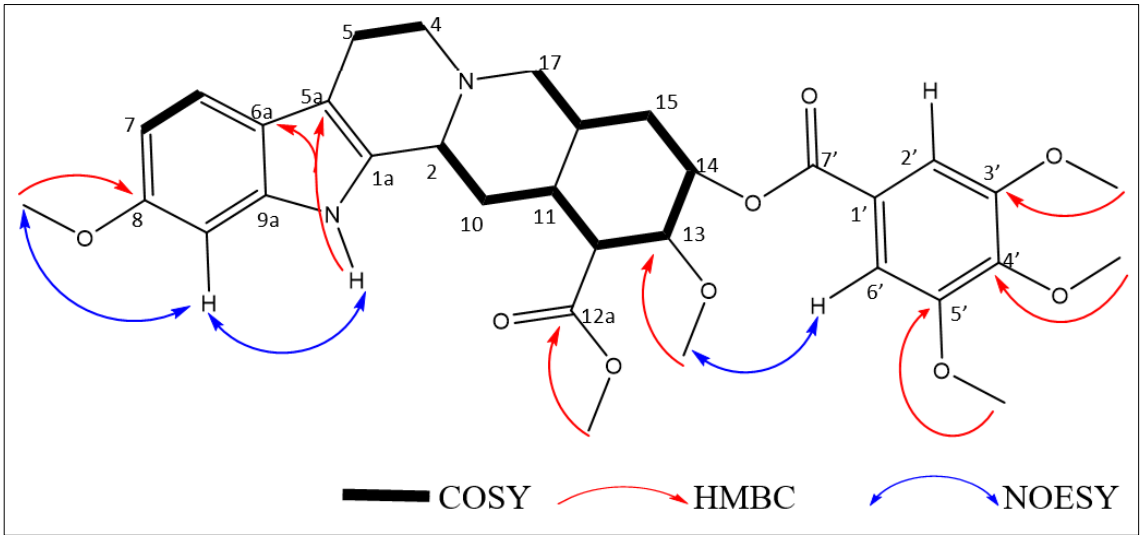

Fig 4: Correlation COSY and HMBC of phytoconstituent (4)

The phytoconstituent (5) 9-ethyl-5-methyl-5a, 6, 8, 9, 10, 11, 11a, 12-octahydro-5H-6, 10:11, 12a-dimethano-indolo[3, 2-b]quinolizin-14, 18-diol (ajmaline) (Figure 1) appears as white crystals (Tf= 206 °C), Rf= 0.35 (CH_2Cl_2/MeOH 15/1), shows a peak in HPLC at Tr= 0.936 min. Its mass spectrum carried out in TOF MS ESI$^+$, shows a peak at m/z 327.21 $[M+H]^+$ corresponding to the gross formula $C_{20}H_{26}N_2O_2$. The ^{1}H NMR spectrum (Table 5) shows signals at 7.43 ppm (1H, d, J = 7 Hz, H7), 6.73 ppm (1H, t, J = 7.4 Hz, H8), 7.10 ppm (1H, t, J = 7.7 Hz, H9), 6.62 ppm (1H, d, J = 7.7 Hz, H10) corresponding to the aromatic ring protons. The spectrum shows a mass of signals at 3.18 ppm (1H, s, H2); 3.45 ppm (1H, d, J = 10 Hz, H3); 3.34 ppm (1H, m, H5); 2.36 ppm (1H, dd, J = 12, 5 Hz, H6a) and 2.14 ppm (1H, m, H6b); 2.35 ppm (1H, dd, J = 14 10 Hz, H11a) and 2.08 ppm (1H, m, H11b); 2.52 ppm (1H, m, H12); 2.48 ppm (1H, m, H13); 4.51 ppm (1H, s, H14); 1.75 ppm (2H, m, H16); 1.48 ppm (1H, m, H17); 4.83 ppm (1H, s, H18). The protons resonating at 1.01 ppm (3H, t, J = 7 Hz, H15) and 2.81 ppm (3H, s) indicate the presence of CH_3- and N-CH_3-. The presence of OH at C14 and C18 is deduced by the chemical shifts of H2 and H5. The ^{13}C NMR spectrum of phytoconstituent (5) indicates 3 quaternary Cs C10a (153.14 ppm), C6a (54.15 ppm) and C7a (129.42 ppm); three secondary carbons C6 (33.182 ppm), C11 (30.07 ppm) and C16 (29.39 ppm). The carbons C7 (122.29 ppm), C8 (120.03 ppm), C9 (129.42 ppm) and C10 (110.20 ppm) characterize the disubstituted aromatic ring; 8 tertiary Cs C2 (75.02 ppm), C3 (54.92 ppm), C5 (54.92 ppm), C12 (27.16 ppm), C13 (45.63 ppm), C14 (76.61 ppm), C17 (25.12 ppm), C18 (88.63 ppm), and 2 primary Cs C15 (11.18 ppm) of the -CH_3 and N-CH_3 group (34.22 ppm). The C14 and C18 carbons are represented by singletons, which distinguishes their R configuration [19].

The phytoconstituent (6) (E)-9-ethylidene-5-methyl-5a,6,8,9,10,11,11a,12-octahydro-5H-6,10:11,12a-dimethanoindolo[3,2-b]quinolizin-14-ol (mauiensine) (Figure 1) a solid as yellow crystals, Rf= 0.13 (CH_2Cl_2/MeOH 15: 1), Tf= 239°C, shows a peak at Tr= 2.83 min in HPLC with the gross formula $C_{20}H_{24}N_2O$ 309.20 $[M+H]^+$. Its ^{13}C NMR spectrum indicates that the molecule also has 20 C just like the phytoconstituent (5) with the difference in chemical shifts of C18 (54.78ppm), C17 (128.70 ppm) and C16 (118.58 ppm) caused by the loss of OH at C18 and the presence of the double bond between C16 and C17. As for the other carbons, their chemical shifts are similar to those of phytoconstituent (5) (Table 5). Similarly, the ^{1}H NMR spectrum of phytoconstituent (6) is identical to that of phytoconstituent (5) except for the presence of proton signals at 3.70 ppm (1H, s, H18a) and 3.60 ppm (1H, s, H18b); of the double bond at 5.35 ppm (1H, q, J = 6.0 Hz, H16). The ^{1}H-^{1}H COSY correlations show the H16 (1H, m, 5.35 ppm) / H15 (3H, t, J = 7 Hz, 1.67 ppm) couplings, as well as the HMBC spectrum shows the H16 (1H, m, 5.35 ppm) / C15 (13.12 ppm, C18 (54.78 ppm) correlations (Table 5). Interpretation of the IR spectrum indicates the same absorption bands as for phytoconstituent (5). Based on the spectral data, and supported with literature data [20, 21], it was concluded that the phytoconstituents (5, 6) are ajmaline and mauiensine respectively (Figure 5).

Table 5: ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), COSY and HMBC of the compounds (5, 6)

N°	Type	δ C		δ H (m, Hz)		COSY		HMBC	
		(5)	(6)	(5)	(6)	(5)	(6)	(5)	(6)
2	CH	75.02	76.40	3,18 (1H, s)	4.47 (1H, s,)			NMe	NMe
3	CH	54.92	57.49	3.45 (1H, d, *J* = 10)	3.28 (1H, d, *J* = 10)	H11	H11		
5	CH	54.92		3.34 (1H, m)	3.20 (1H, m)	H6	H6		
6	CH_2	33.182	33.81	2.36 (1H, dd, *J* = 12, 5, H6a) 2.14 (1H, m, H6b)	2.31 (d, 6.0, 1H, Ha) 2.42 (dd, 7.0, 3.1, 1H, Hb	H5	H5		
6a	C	54.15	53.48						
7	CH	122.29	123.23	7,43 (1H, d, *J* = 7)	7.45 (d, J = 7.3 Hz, 1H),	H8	H8	10a, 7a	10a, 7a
7a	C	129.42	130.04						
8	CH	120.03	120.28	6.73 (1H, t, *J* = 7.4)	6.77 (dd, J = 7.8, 3.2, 1H	H7, H9	H7, H9	7a, 10	7a, 10
9	CH	129.42	127.93	7.10 (1H, t, *J* = 7.7)	7.15 (d, J = 8.2, 1H),	H8, H10	H8, H10	10a, 7, 10	10a, 7, 10
10	CH	110.20	110.09	6.62 (1H, d, *J* = 7.7)	6.65 ppm (1H, d, J = 7.7)	H9	H9		
10a	C	153.14	153.48						
11	CH_2	30.07	29.07	2.35 (1H, dd, *J* = 14 10, H11a) 2.08 (1H, m, H11b)	1.94 (d, 6.0, 1H, H11a) 2.02 (dd, 7.0, 3.1, 1H, H11b)	H3, H12	H3, H12		
12	CH	27.16	26.99	2.52 (m, 1H)					
13	CH	45.63	75.44	2.48 (1H, m)	3.14 (m, 1H)	H14	H14		
14	CH	76.61	78.21	4.51 (1H, s)	4.81 (s, 1H)	H13	H13		
15	CH_3	11.88	13.12	1.01 (3H, t, *J* = 7)	1.67 (d, *J* = 6.7, 3H).			16	
16	CH_2	29.39	118.58	1.75 (1H, m, H16)	5.35 (q, J = 6.0, 1H),	H15	H15		15, 18
17	CH	25.12		1.48 (m, 1H)		H16, H18			
17	C		128.70						
18	CH	88.3	54.78	4.83 (1H, s, H18)	3.70 (1H, s, H18a) 3.60 (1H, s, H18b)	H17			
	NCH_3	34.22	34.70	2.81 (3H, s)	2.80 (s, 3H)			10a; 2	10a, 2

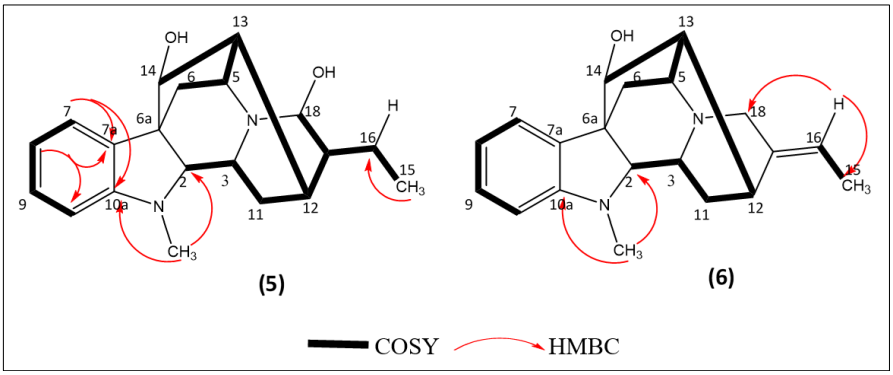

Fig 5: Correlations COSY and HMBC of phytoconstituents (5, 6)

In order to better identify the structure-activity relationship, in our study, the antioxidant and anticholinesterase activity of the isolated phytoconstituents (1-5) were evaluated.

3.3. Antioxidant activity of isolated phytoconstituents

Figure 6 shows the percent reductions (PR) of the DPPH radical.

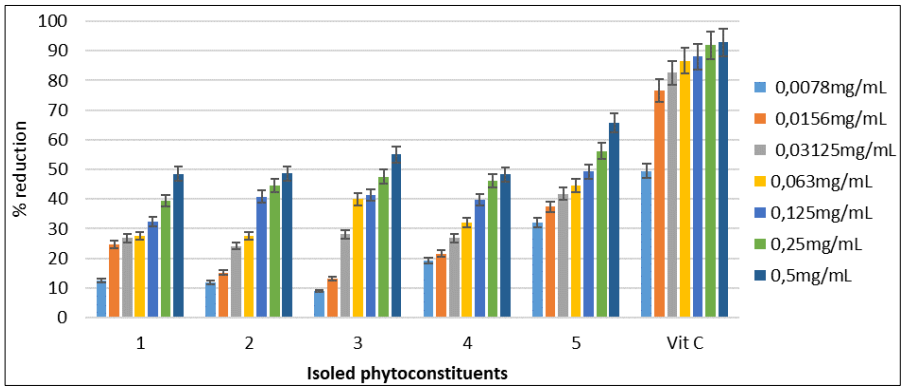

Fig 6: Percentages of reduction of DPPH by isolated phytoconstituents.

1: Methyl 3, 4, 5-trimethoxybenzoate; 2: methyl (E)-3-(3, 4, 5-trimethoxyphenyl)acrylate; 3: 1-carbomethoxy-β-carboline Methyl; 4: Reserpine; 5: Ajmaline; Vit C: vitamin C

The PR of the DPPH radical varies with the concentrations tested. This seems to show therefore a concentration-dependent effect. However, compared to vitamin C, the phytoconstituents analyzed showed overall a very low antioxidant activity (AO) with PR ≤ 50%. Phytoconstituent (1) showed no ability to reduce the DPPH radical (PR ≤ 50%), corroborating the results reported in the literature [22].

Phytoconstituents (2, 4) showed the same DPPH reduction profile as phytoconstituent (1). They therefore lacked hydrogen (H) scavenging activity of DPPH, which seems to correlate well with their molecular structures containing no labile H. On the other hand, phytoconstituent (3) has a DPPH reduction capacity (PR = 55.10%) at 0.5 mg/mL, as does phytoconstituent (5) (PR = 65.71%) and (PR = 65.71%) at 0.5 mg/mL and 0.25 mg/mL, respectively. For the purpose of assessing their antioxidant efficiency (AOE) with respect to DPPH, the CR_{50} was determined (Table 6). The lower this parameter, the more pronounced the AOE [9, 10]. The CR_{50} of phytoconstituents (3, 5) with respective values of 0.346 mg/mL and 0.127 mg/mL are significantly higher than that of vitamin C (0.003 mg/mL) (Table 6). From the results, it is evident that phytoconstituent (5) is clearly more antioxidant than phytoconstituent (3). Oxidative stress is the set of aggressions caused to the organism by radical substances derived from oxygen and nitrogen. This biological phenomenon is incriminated in the pathogenic etiology [23, 24]. It would even potentiate neurodegenerative pathologies (Parkinson and Alzheimer diseases). And only antioxidants are essential for slowing down cellular oxidation, and thus maintaining health. Zhang *et al.*, [5] reported the anticholinesterase activity of indolic monoterpene alkaloids extracted from the stems of R. vomitoria. Therefore, the root of *Rauvolfia vomitoria* is used in the treatment of mental diseases in indigenous medicine. For this reason, we evaluated the anticholinesterase potential of phytoconstituents isolated from the bark of its root, which present a chemical diversity.

Table 6: CR_{50} of isolated phytoconstituents and vitamin C

Phytoconstituant	1	2	3	4	5	Vit C
CR_{50} (mg/mL)	ND	ND	0,346	ND	0,127	0,003

ND: not determined; 1: Methyl 3, 4, 5-trimethoxybenzoate; 2: methyl (E)-3-(3, 4, 5-trimethoxyphenyl)acrylate; 3: 1-carbomethoxy-β-carboline Methyl; 4: Reserpine; 5: Ajmaline; Vit C: vitamin C

3.4. Anticholinesterase activity of isolated phytoconstituents

The inhibitory activity of phytoconstituents was evaluated against AChE for 360 s according to Ellman [11]. Galantamine was used as the reference anticholinesterase for the positive control. Figure 7 shows the results obtained. The percentages of inhibition (PI) fluctuate with concentration and time.

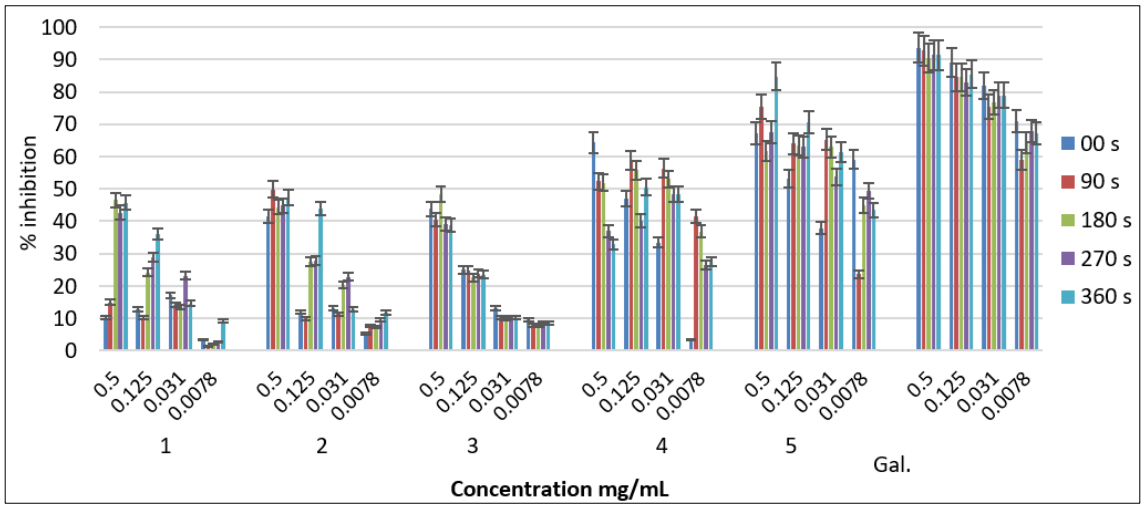

Fig 7: Percentage of AChE inhibition by isolated phytoconstituents

The isolated phytoconstituents showed variable PIs. Indeed, phytoconstituents (1), (2), and (3) showed no ability to inhibit AChE (PI << 50%) for 360 s at all concentrations tested. Phytoconstituent (4) inhibited AChE at 0.5 mg/mL (PI = 64.39% at 0 s; 52.36% at 90 s and 51.93% at 180 s). Furthermore, inhibition of AChE by said phytoconstituent is found at 0.125 mg/mL (PI=58.79% at 90 s, 55.79% at 180 s) and 0.3125 mg/mL (PI= 56.38% at 90 s, 53.01% at 180 s). As for phytoconstituent (5), it exhibited the best PI at 0.5 - 0.03125 mg/mL precisely at 360 s (PI= 84.82; 70.67 and 61.37%); 270 s (PI= 67.58; 62.95 and 53.71%); 180 s (PI= 61.65; 63.50 and 62.97%) and 90 s (PI= 75.52; 54.03 and 65.26%). Phytoconstituent (5) exhibited the best anticholinesterase activity compared to galantamine (PI> 80%). This phytoconstituent would govern the anticholinesterase virtue conferred on the plant. Thus, is suggested an explanatory pathway for the use of the root of *Rauvolfia vomitoria* in traditional pharmacopoeia in the treatment of mental diseases.

4. Conclusion

Phytochemical and biological investigations of the chloroform extract of *Rauvolfia vomitoria* root bark were carried out. The methyl esters of 3, 4, 5-trimethoxybenzoic and 3, 4, 5-trimethoxycinnamic acids and the indole type alkaloids of which 3 known (reserpine, ajmaline and mauiensine) and another 1-carbomethoxy-β-carboline) were isolated from the root bark of the Ivorian species for the first time. Their molecular structures were successfully elucidated by MS, NMR (^{1}H, ^{13}C), IR, UV, and melting points determined. The combined antioxidant and anticholinesterase activities of the phytoconstituent (5) propose the utility of *R. vomitoria* in phytotherapy through the development of phytoremedies.

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6. Conflict of Interest
None

7. References

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