Original Research Article

ISOLATION, CHARACTERIZATION AND EVALUATION OF THE ANTIOXIDANT POTENTIAL OF DIFFERENT EXTRACTS AND BIOMOLECULES OF THE ROOTS OF *PAULLINIA PINNATA* (Sapindaceae)

Abstract

Cardiovascular diseases, which are now among the leading causes of death in the world, are increasingly prompting the search for new antioxidant remedies. It is in this context that the chemical study and evaluation of the antioxidant potential of the different extracts of *Paullinia pinnata* (Sapindaceae), a plant used in the traditional treatment of cardiovascular diseases was carried out. The assessment of antioxidant potential was carried out by the ABTS free radical scavenging method. Phytochemical study of extracts (ethyl and aqueous acetate) of *Paullinia pinnata* roots isolated epicatechin, laproanthocyanidin A-2, epicatechin-(2β→O→7, 4β→8)-epicatechin-(4β→8)-epicatechin (cinnamtannin B-1) and O-β-D-glucopyranosyloxy-4-methyl-2(5H)-furanone. These compounds were first isolated from the genus Paullinia pinnata from Côte d'Ivoire. Their structures are elucidated by spectroscopic analyses (NMR and MS) in comparison with data from the literature.

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Keywords: Cardiovascular diseases, *Paullinia pinnata*, antioxidant potential.

Introduction :

Cardiovascular diseases, a set of disorders affecting the heart and blood vessels, are responsible for many public health problems and are now among the leading causes of death in the world.[1] To address this problem, World Health Organization (WHO) encourages African countries to develop regional strategies that take into account traditional medicine, in order to undertake research on medicinal plants and promote their optimal uses in health care delivery systems.[2] Medicinal plants have always been an essential source for the discovery of new medicines.[3] Indeed, they contain compounds with pharmacological properties (antioxidant, hypertensive, antidiabetic etc ...) that act directly on the body[4] and provide appreciable healing effects that no synthetic and combinatorial chemistry can offer us. As a result, new antioxidant molecules of natural origin are sought[5] for the prevention and treatment of cardiovascular diseases.[5] It is in this context that a recent study carried out on plants with cardiovascular properties in Côte d'Ivoire showed that *Paullinia pinnata* possessed good antioxidant activity and a high content of total polyphenol.[6] Given the many therapeutic

virtues of *Paullinia pinnata*,[7-8-9-10] it is important to deepen phytochemical studies. The present study aims on the one hand to isolate and characterize biomolecules extracts of the roots of *Paullinia pinnata* from Côte d'Ivoire and on the other hand to evaluate the antioxidant potential by the method of scavenging free radicals of ABTS of the different extracts and biomolecules isolated in order to justify its use in traditional medicine.

EXPERIMENTAL METHOD

Analysis by thin-layer chromatography was performed on plates of Silicagel 60 F254 (Merck). After development in glass cells, the plates were revealed using an ultraviolet lamp at 2 wavelengths (254 nm and 366 nm) and with a solution of sulfuric acid. The separation of the compounds was performed on an open column of Merck silica gel 40-63 µm and under reduced pressure with grafted silica RP-C18. Purification was done on sephadex LH-20 gel column. The mass spectra of the isolated compounds were performed by electrospray ionization in positive mode, using a micrO-TOF-Q-II 10300 mass spectrometer equipped with an ESI source. The 1D and 2D NMR spectra were recorded using a Bruker Avance 600 MHz spectrometer. Chemical displacements are expressed in ppm. The deuterated solvent used is CD_3OD (δ_H= 3.31ppm ; δ_C= 49.00 ppm) and D_2O (δ_H= 4.79 ppm). The method of scavenging free radicals of ABTS permit to evaluate the antioxidant potential of the different extracts and biomolecules isolated

Comment [TS2]: How the Plant was authenticated for the confirmation of the species? Details to be provided with the support of plant taxonomist.

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RESULTS AND DISCUSSION

Determination of AJPP1, AJPP2 and AJPP3 compound

The compound AJPP1 is present in the form of a solid, soluble in methanol (CH_3OH). The 1H NMR and 13C NMR spectra were realized in deuterated methanol (CD_3OD). Its low-resolution mass spectrum (ESI-MS) indicates the peak of the molecular ion $[M-H]^+$ at m/z = 289.1 Which gives an exact mass of 290.1 g/mol. This mass corresponds to the general formula $C_{15}H_{14}O_6$. The IR spectrum has a wide absorption band characteristic of the alcohol function υ_{OH} = 3271cm^{-1}, characteristic bands at the intensities of the aromatic nuclei $\upsilon_{C=C}$ = 1452-1610 cm^{-1} and a characteristic band of the ether function υ_{C-O} 1062cm^{-1}. The characteristics 1H NMR and 13C NMR are reported in Table 1. The 1H NMR spectrum at 600 MHz in the CD_3OD (Figure 2) shows characteristics of a flavonoid-type skeleton[11] that can be identified by: a doublet at δ_H = 6.97 ppm (J = 2 Hz) corresponding to the proton H-2', a doublet split at δ_H = 6.80 ppm (J = 8.3/ 2 Hz) corresponding to the proton H-6' and a doublet at δ_H = 6.76 ppm (J = 8.3 Hz) corresponding to the proton H-5'. These three signals have an

ABX coupling system characteristic of the B-ring of a flavonoid. There is also a doublet at δ_H = 5.92 ppm in the form of a doublet (J = 2.3 Hz) corresponding to the proton H-8 a doublet at δ_H = 5.94 ppm in the form of a doublet (J = 2.3 Hz) corresponding to the proton H-6. These two signals have an AB coupling system characteristic of the A cycle of a flavonoid. We then observe a singlet at δ_H = 4.85 corresponding to the proton H-2 and a multiplet at δ_H = 4.21 corresponding to the proton H-3. Then 3 massifs, in the form of a doublet split at δ_H = 3.34 ppm (J = 16.4/ 4.6 Hz) corresponding to the proton H-4 and a doublet doubled at δ_H = 2.77 ppm (J = 16.2/ 3.1 Hz) corresponding to the second proton H-4. The 13C NMR spectrum (Figure 3) indicates the presence of fifteen (15) carbons characteristic of a flavonoid including two (2) aromatic quaternary carbons (C-1' and C-10), five (5) oxygenated aromatic quaternary carbons (C3', C4', C5, C7 and C9) five (5) aromatic CH carbons (C-8, C-6, C-2', C-5' and C-6'), two oxygenated CH carbons (C-2 and C-3) and one CH_2 carbon. Chemical displacements are observed at δ_C = 95.89 ppm and δ_C = 96.40 ppm corresponding to the aromatic CH of the A ring (C-8 and C-6) respectively. Also at δ_C= 119.39 ppm, δ_C= 115.33 ppm and, δ_C= 115.89 ppm correspond respectively to the CH of the B cycle (C-2', C-5' and C-6'). There is the presence of a peak at δ_C = 100.08 ppm attributed to carbon C-10; at δ_C= 132.29 ppm assigned to carbon C-1', signal at δ_C= 145.80, δ_C= 145.97 ppm assigned respectively to carbons C-3' and C-4' signals at δ_C= 157.39 ppm, δ_C= 157.39 ppm and δ_C= 158.02 ppm are attributed respectively to carbons C-5, C-7 and C-9. The COSY spectrum has two protons not equivalent to δ_H= 2.74 (dd, J = 16.6/ 3.12 Hz) and δ_H= 2.84 (dd, J = 16.7/ 4.7Hz) corresponding to the CH_2 of the C cycle that correlate with the proton H-3 at δ_H= 4.2 ppm and weakly with the proton H-2 at δ_H= 4.82 ppm. Correlations are observed between protons H-2 and H-3, also between protons H-5' and H-6'. The HMBC spectrum makes it possible to establish correlations between the H-2 proton and the C-1', C-2'and C-5' carbons, also between the H-4 and the C-3, C-2, C-10 carbons. The results of spectral analyses in 1D (1H, 13C) and 2D NMR (COSY, HSQC, HMBC and NOESY) can be compared to that of the literature.[12] The compound AJPP1 is most likely an epicatechin. The cis position of protons H-2 (δ_H = 4.82 J = 5.0 Hz) and H-3 (δ_H = 4.2 ppm) was deduced by the value of their low coupling constant which is 5 Hz.[13-14-15-] On the other hand, this coupling constant is about 7-10 Hz in catechin.[13-14-15] The compound AJPP1 is therefore epicatechin (Figure 1) since, this is also confirmed by the value of the chemical displacement of carbon C-2 observed at 79.90 ppm. It is known that, in epicatechin, the chemical displacement of carbon C-2 is slightly less than 80 ppm while this carbon resonates slightly above 80 ppm in catechin. [14-15-16-17] The NOE effects observed on the NOESY spectrum between H-2/H-3 confirm the stereochemistry

of epicatechin by the presence of NOE correlation between these two protons. In catechin, the two protons of the pyranic ring are in the trans position and they do not show NOE effects between them.[15-18] This compound is reported for the first time in the genus Paullinia.

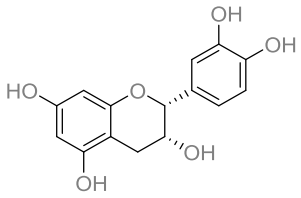

Figure 1 : Structure of the molecule of epicatechin (AJPP 1)

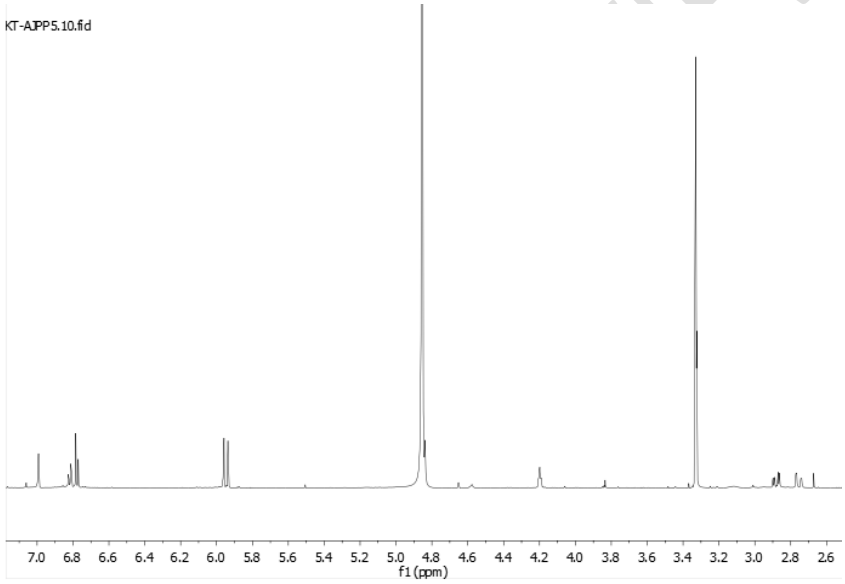

Figure 2: 1H-NMR Spectrum of epicatechin (AJPP 1)

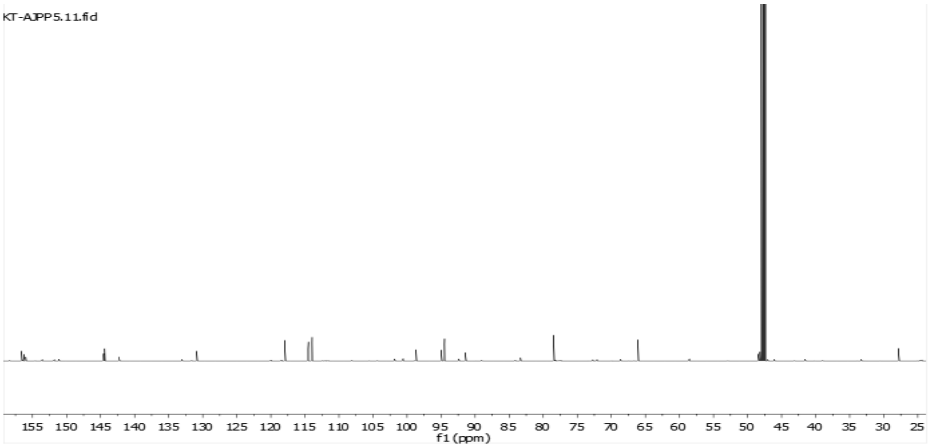

Figure 3 : 13C-NMR Spectrum of epicatechin (AJPP 1)

Table 1: Proton and Carbon NMR signals of epicatechin (AJPP 1)

Position	^{1}H NMR δ (ppm), J to Hz	^{13}C NMR δ (ppm)	J mod
2	4.82 (d, J=5.0 Hz)	79.90	CH
3	4.2 (m)	67.50	CH
4	2.74 (dd, J = 16.6/ 3.1 Hz)	29.27	CH_2
5	2.86 (dd, J = 16.6/ 4.7 Hz)	157.70	C
6	-	96.40	CH
7	5.94 (d, J = 2.3 Hz)	157.39	C
8		95.89	CH
9	5.92 (d, J = 2.3 Hz)	158.02	C
10	-	100.08	C
1’	-	132.29	C
2’	-	119.39	CH
3’	6.97 (d, J = 2 Hz)	145.80	C
4’	-	145.97	C
5’	-	115.33	CH
6’	6.76 (d, J = 8.3 Hz)	115.89	CH

The compound AJPP2 is a pale yellow solid, soluble in methanol (CH_3OH). The 1H NMR and 13C NMR spectra were realized in deuterated methanol (CD_3OD). The IR spectrum has a

wide absorption band characteristic of the alcohol function υ_{OH} = 3390 cm^{-1}, characteristic bands at the intensities of the aromatic nuclei $\upsilon_{C=C}$ = 1450-1616 cm^{-1} and a characteristic band of the ether function υ_{C-O} = 1072cm^{-1}. Its low-resolution mass spectrum (ESI-MS) indicates the peak of the pseudo-molecular ion $[M+Na]^+$ at m/z = 599.4 which gives an exact mass of 576.4 g/mol. This mass corresponds to the gross formula $C_{30}H_{23}O_{12}$ suggesting two units of flavan-3-ol with the existence of a bond of type A (double interflavanic bond).[19] Indeed, the molecular weight of two units of flavan 3-ol with simple interflavanic bonds (type B), would be 578 g/mol.[20] It is therefore a proanthocyanidin dimer type A. To facilitate the understanding of NMR attributions, we designate the two flavanolic units as T and Q as follows.

The characteristics 1H NMR and 13C NMR are reported in Table 2. The 1H NMR spectrum (Figure 5) shows two zones of aromatic protons, one zone (from δ_H = 7.14ppm to δ_H = 6.81 ppm) corresponding to the ABX coupling attributed to the protons of the B and E cycles successively of the T and Q monomers followed by another zone (from δ_H = 6.01 ppm to δ_H = 6.01 ppm) corresponding to the AB coupling for two meta-coupled protons assigned to the protons of the A and D cycles successively of the T and Q monomers. This same spectrum has an aliphatic zone corresponding to the protons of the C and F cycles successively of the T and Q monomers. The 13C NMR spectrum (Figure 6) indicates the presence of thirty (30) carbons characteristic of a flavan-3-ol dimer including ten (10) oxygenated aromatic quaternary carbons, five (5) quaternary carbons, nine (9) aromatic CH carbons, three (3) oxygen CH carbons, one (1) non-oxygenated CH carbon, one (1) oxygenated quaternary carbon, and one (1) CH_2 carbon. Chemical displacements are observed at δ_C = 98.32 ppm, δ_C = 96.64 ppm δ_C = 96.52 ppm and δ_C = 107.27 ppm corresponding to the aromatic CH of the A ring of the T monomer (C-6 and C-8) and the F ring of the Q monomer (C-6 and C-8) respectively. Also at δ_C = 115.68 ppm, δ_C = 116.06 ppm and, δ_C= 119.76 ppm, δ_C = 115.95 ppm, δ_C= 115.64 ppm and, δ_C= 120.38 ppm, correspond respectively to the CH of the B cycles of the T monomer (C-2', C-5' and C-6') and the E ring of the Q monomer (C-2', C-5' and C-6'). There are two peaks at δ_C = 104.28 ppm and δ_C = 102.44 ppm attributed respectively to carbon C-10T of the A cycle and C-10Q of the A cycle. The signal at δ_C = 132.46 ppm attributed to carbon C-1'T and that at δC = 131.20 ppm attributed to carbon C-1'Q. Chemical displacements at δ_C = 145.65 ppm, δ_C = 146.77 ppm, δ_C = 146.0 ppm and δ_C = 146.30 ppm attributed respectively to carbons C-3'T, C-4'T of the B cycle, C-3'Q and C-4'Q of the E cycle. Signals at δ_C = 157.0 ppm, δ_C = 158.13 ppm, δ_C = 154.25ppm, δ_C = 156.60 ppm, δ_C = 152.30 ppm and δ_C = 152.13 ppm are attributed respectively to the C-5T, C-7T, C-

9T carbons of the A cycle of the T monomer and C-5Q, C-7Q and C-9Q of the D cycle of the Q monomer. The COSY spectrum has two protons not equivalent to δ_H = 2.76 [dd,H-4Q, J = 17.2/ 2.3 Hz], δH = 2.95 [dd,H-4Q, J = 17.2 / 4.9 Hz] corresponding to the CH_2 of the F cycle of the Q monomer that correlate with the proton H-3Q at δ_H=4.2 ppm of the T monomer and C-5Q, C-7Q and C-9Q of the D cycle of the Q monomer. The COSY spectrum has two protons not equivalent to δ_H = 2.76 [dd,H-4Q, J = 17.2/ 2.3 Hz], δ_H = 2.95 [dd,H-4Q, J = 17.2 / 4.9 Hz] corresponding to the CH_2 of the F cycle of the Q monomer that correlate with the proton H-3Q at δ_H = 4.2 ppm and weakly with the proton H-2Q at δ_H = 4.92 ppm. There is a correlation between protons H-4T and H-3T, between H-2Q and H-2'Q. Correlation tasks are observed between H-2'Q and H-6'Q, H-5'Q and H-6'Q and also between H-2'T and H-6'T, H-5'T and H-6'T. The HMBC spectrum makes it possible to establish correlations between the H-2Q proton and the C-4Q, C-1'Q, C-2'Q and C-6'Q carbons and weakly with the C-7Q and C-9Q carbons. Correlation tasks are observed between the proton H-4T and the carbons C-3T, C-2T, C-7Q, C-8Q, C-9Q and C-10Q. By comparing the chemical displacement of carbon C-2T (δC = 100.19 ppm) to that of the compound AJPP1 (δC = 79.90 ppm), we observe a de-icing of carbon C-2 suggesting that this carbon is bound to two oxygens. Shielding carbon C-7Q (δC = 152.30 ppm) against carbon C-7T assumes that the monomer T is connected to the monomer Q with a second bond of type (2→O→7).[14-21-22] The junction between the two units of flavan-3-ol is justified by the HMBC correlation between the proton H-4T and the carbon C-8Q. There are also correlations between H-4T and carbons C-2T, C-3T, C-7Q, C-9Q and C-10Q. The results of the 1D (1H, 13C) and 2D (COSY, HSQC, HMBC and NOESY) NMR spectral analyses compared to the data in the literature[23] identified the compound AJPP2 as proanthocyanidin A-2 (Figure 4). This compound was first isolated in the genus Paullinia.

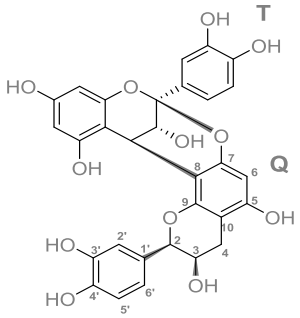

Figure 4 : Structure of the molecule of proanthocyanidin A-2 (AJPP 2)

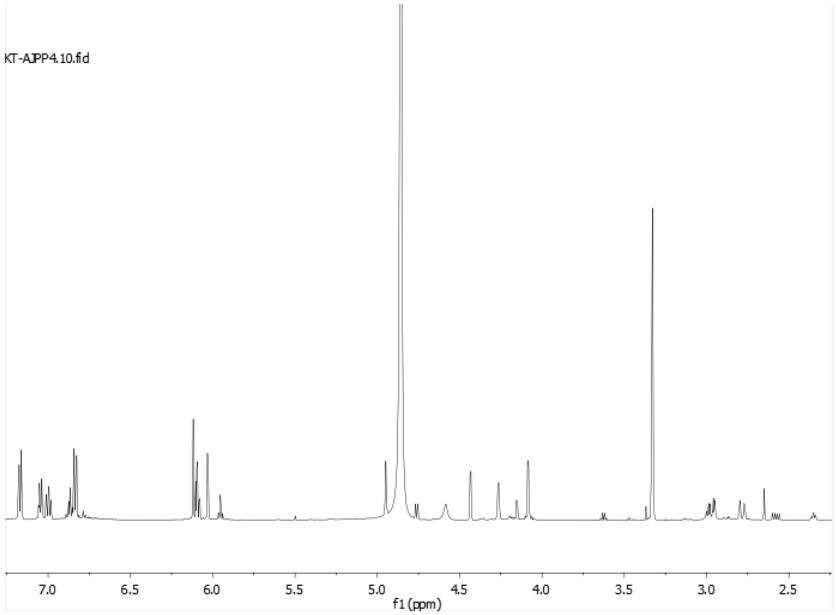

Figure 5 : 1H NMR spectrum of proanthocyanidin A-2 (AJPP 2)

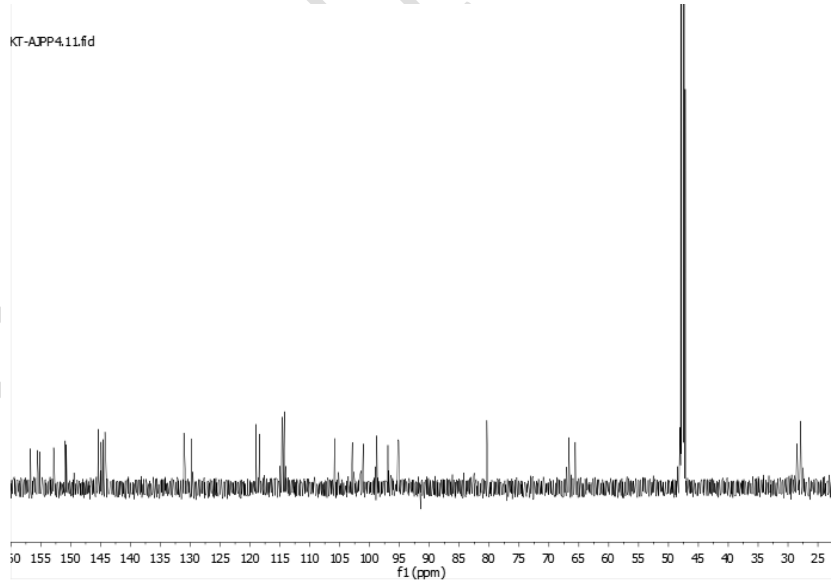

Figure 6 : 13C NMR spectrum of proanthocyanidin A-2 (AJPP 2)

Table 2: Proton and carbon NMR signals of proanthocyanidin A-2

Position	^{1}H NMR δ (ppm), J to Hz	^{13}C NMR δ (ppm)	J mod
C-2T	-	41.19	C
C-3T	4.06 (d, J = 3.4)	68.08	CH
C-4T	4.41 (d, J = 3.4)	29.26	CH
C-5T	-	157.00	C
C-6T	6.01 (d, J = 2.4)	98.32	CH
C-7T	-	158.13	C
C-8T	6.07 (d, J = 2.4)	96.64	CH
C-9T	-	154.25	C
C-10T	-	104.28	C
C-1'T	-	132.46	C
C-2'T	7.14 (d, J = 2.2)	115.68	CH
C-3'T	-	145.65	C
C-4'T	-	146.77	C
C-5'T	6.81 (d, J = 8.3)	116.06	CH
C-6'T	7.02 (dd, J = 8.3/2.2)	119.76	CH
C-2Q	4.92 (brs)	81.77	CH
C-3Q	4.24 (m)	66.98	CH
C-4Q	2.74 (dd, J = 17.2/4.9)	29.90	CH_2
C-5Q	2.84 (dd, J = 17.2/2.3)	156.60	C
C-6Q	-	96.52	CH
C-7Q	6.10 (s)	152.30	C
C-8Q	-	107.23	C
C-9Q	-	152.13	C
C-10Q	-	102.44	C
C-1'Q	-	131.20	C
C-2'Q	-	115.95	CH
C-3'Q	7.15 (d, J = 2.1)	146.00	C
C-4'Q	-	146.30	C
C-5'Q	-	115.64	CH
C-6'Q	6.81 (d, J = 8.2)	120.38	CH

The compound AJPP3 is in the form of a white solid, soluble in methanol (CH_3OH). The NMR spectra 1H and 13C were realized in deuterated methanol (CD_3OD). Its high-resolution mass spectrum (ESI-HR) indicates the peak of the pseudo-molecular ion $[M+H]^+$ at m/z = 865.19 which gives an exact mass of 864.19 g/mol. This mass corresponds to the raw formula $C_{45}H_{36}O_{18}$. The molecular weight of AJPP3 corresponds to the condensation of three units of flavan-3-ol and assumes the existence of a type A bond (double interflavanic bond).[19] Indeed, the molecular weight of three units of flavan-3-ol with simple interflavanic bonds (type B), would be 866 g / mol.[20] It is therefore a proanthocyanidin trimer containing a type A bond and a type B bond. To facilitate the understanding of NMR attributions, we designate the three flavanolic units as follows by T, Q and M. These three units are characteristic of a trimer having rings A, B, C for the monomer T, the cycles D, E, F for the monomer Q and the rings G, H, I for the monomer M. The NMR characteristics 1H and 13C are reported in Table 3. The 1H NMR spectrum (Figure 8) shows three zones of aromatic protons. A zone (from δ_H = 7.34 ppm to δ_H = 6.75 ppm) corresponding to the ABX coupling assigned to the protons of the B, E and H cycles respectively of the monomers T, Q and M, characteristic of 3′, 4′-disubstituted protons of flavan-3-ol followed by another zone (from δ_H= 6.11ppm to δ_H= 5.71ppm) corresponding to the AB coupling for three meta-coupled protons assigned to the protons of the A cycles, D and G respectively of the monomers T, Q and M. This same spectrum has an aliphatic zone corresponding to the protons of the C, F and I cycles respectively of the monomers T, Q and M. The NMR spectrum 13C (Figure 9) indicates the presence of forty-five (45) carbons characteristic of a trimer of flavan-3-ol including fifteen (15) oxygenated aromatic quaternary carbons, eight (8) quaternary carbons, thirteen (13) aromatic CH carbons, five (5) ch oxygen carbons, two (2) non-oxygen CH carbons, one (1) oxygenated quaternary carbons and one (1) CH_2 carbon. On the COSY spectrum, correlations are observed between protons H-3T and H-4T, H-4Q and H-3Q, H-2Q and H-3Q, H-2Q and H-4Q, H-4M and H-3M. The HMBC spectrum makes it possible to establish correlations between the proton H-2Q and the carbons C-1'Q, C-2'Q and C-6'Q. Correlations are observed between proton H-6Q and carbons C-5Q, C-7Q, C-8Q and C-10Q. Correlation tasks are observed between the proton H-4T and the carbons C-2T, C-3T, C-5T, C-9T, C-10T. Relative to the monomers Q and M, we observe that the proton H-2T is absent. In addition, by comparing the chemical displacements of carbon C-2Q (δ_C = 78.84 ppm) and C-2M (δ_C = 80.27 ppm) to that of C-2T (δ_C = 99.92 ppm) we observe a de-icing of carbon C-2T suggesting that this carbon is bound to two oxygens. Shielding carbon C-7Q (δ_C= 151.06 ppm) against carbon C-7T (δ_C= 157.80 ppm) assumes that the monomer T is connected to the

monomer Q with a second bond of type (2→O→7).[14-21-22] Carbon C-4Q (δC = 38.26 ppm) is deblindered by about 8 ppm compared to C-4M (δC = 29.84 ppm), following its substitution by the aromatic ring of the G ring of the monomer M. The correlations observed in HMBC between the proton H-4Q and the carbons C-7M, confirm the nature of the bond (4→8) between the two flavanolic units. On the COSY spectrum there is a coupling between the proton H-4Q and the proton H-3Q (δ_H = 4.13, d, J = 1.43 Hz) itself coupled with the proton H-2 Q (δ_H = 5.72 ppm, brs). There is a low coupling constant between protons H-2Q and H-3Q (J H2-H3 = 1.43 Hz) and the chemical displacement of carbon C-2Q at 78.84 ppm (< 80 ppm) show that the monomer Q is an epicatechin. The junction between the three units of flavan-3-ol is justified by the HMBC correlation between proton H-F4 and carbon C-G8, as well as proton H-C4 and carbon C-D8. The result of spectral analyses in 1D NMR (1H, 13C) and 2D COSY, HSQC, HMBC) compared with data from the literature[23] a identifies the compound AJPP3 as epicatechin-(2β→O→7, 4β→8)-epicatechin-(4β→8)-epiccatechin (cinnamtannin B-1) (Figure 7). This compound was first isolated in 1983 in Ceylon cinnamon, *Cinnamomumzeylanicum* (Lauraceae).[24]

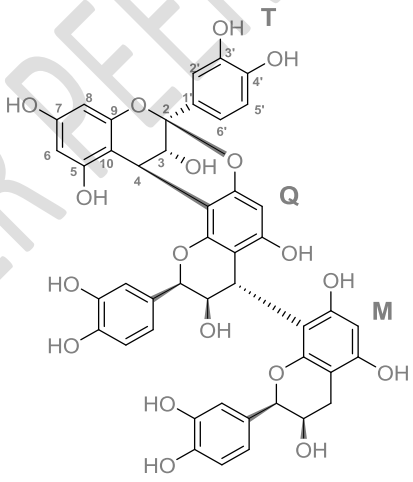

Figure 7 : Structure of the molecule of cinnamtannin B-1

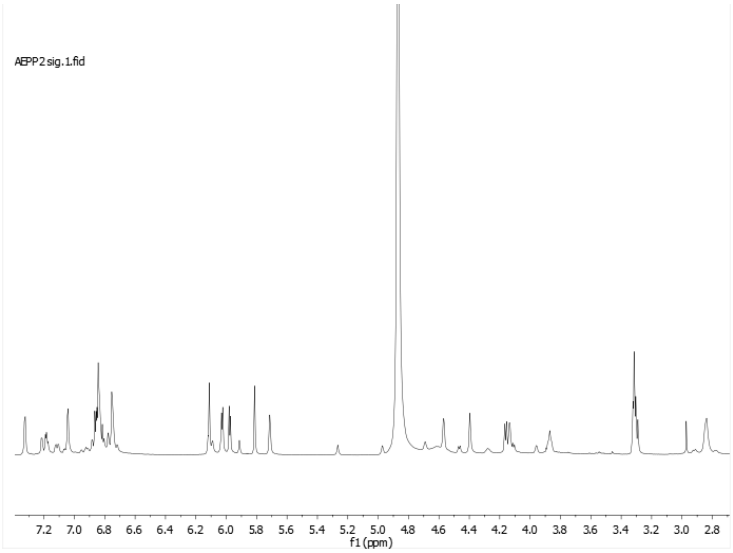

Figure 8 : 1H NMR- spectrum of cinnamtannin B-1 (AJPP 3)

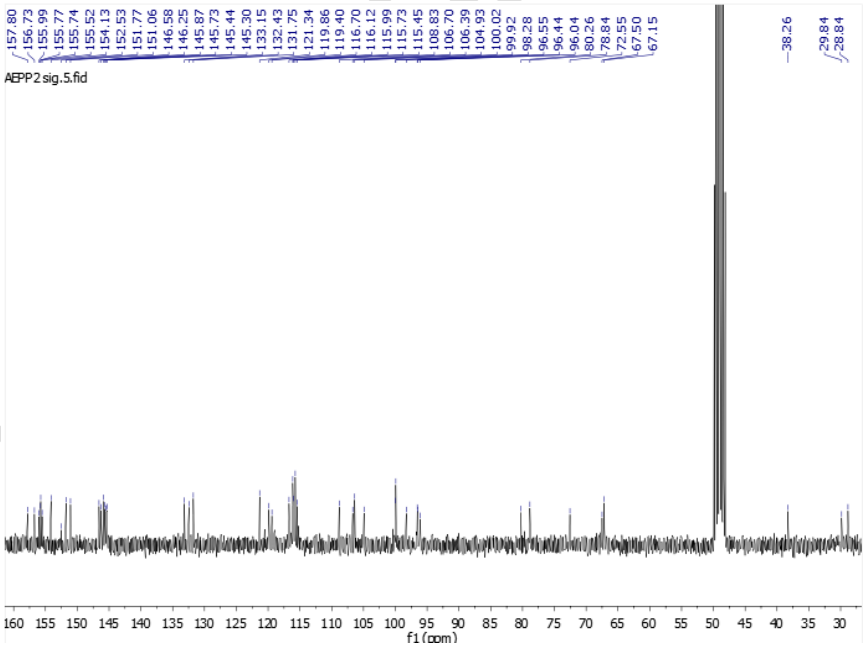

Figure 9 : 13C NMR- spectrum of cinnamtannin B-1 (AJPP 3)

Table 3 : Proton and carbon NMR signals of cinnamtannin B-1 (AJPP 3)

Position	^{1}H NMR δ (ppm), J to Hz	^{13}C NMR δ (ppm)	J mod
C-2T	-	99.92	C
C-3T	3.29 (d, J = 3.5)	67.15	CH
C-4T	4.18 (d, J = 3.5)	28.84	CH
C-5T	-	156,76	C
C-6T	5.98 (d, J = 2.4)	98.28	CH
C-7T	-	157.80	C
C-8T	6.03 (d, J = 2.3)	96.44	CH
C-9T	-	154.13	C
C-10T	-	104.93	C
C-1'T	-	132.43	C
C-2'T	7.02 (d, J = 1.7 Hz)	115.73	CH
C-3'T	-	145.44	C
C-4'T	-	146.58	C
C-5'T	6.85	116.12	CH
C-6'T	6.86	119.86	CH
C-2Q	5.71 (brs)	78.84	CH
C-3Q	4.13 (d, J = 1.4)	72.53	CH
C-4Q	4.59 (brs)	38.26	CH_2
C-5Q	-	155.74	C
C-6Q	5.8 (s)	96.04	CH
C-7Q	-	151.06	C
C-8Q	-	106,39	C
C-9Q	-	151,73	C
C-10Q	-	106.70	C
C-1'Q	-	131.75	C
C-2'Q	7.33 (d, J = 1.99)	116.70	CH
C-3'Q	-	145.87	C
C-4'Q	-	146.26	C
C-5'Q	7.19 (d J = 1.74)	115.73	CH
C-6'Q	7.2 (dd, J = 8.3/2.1)	121.34	CH
C-2M	4.40 (s)	80,27	CH
C-3M	3.87	67.50	CH
C-4M	2.85	29.84	CH_2
C-5M	-	155.99	C
C-6M	6.11 (s)	96.55	CH
C-7M	-	155.52	CH
C-8M		108.83	C
C-9M	7.13 (dd, J = 5.1/1.6)	155.78	C
C-10M		100.02	C
C-1'M	6.77	133.15	C
C-2'M	6.75	115.46	CH
C-3'M	3.29 (d, J = 3.5)	145.30	C
C-4'M	4.18 (d, J = 3.5)	145.73	C
C-5'M	-	115.99	CH
C-6'M	5.98 (d, J = 2.4)	119.40	CH

The compound AJPP4 was isolated as a yellow solid, soluble in dudimethylsulfoxide (DMSO). The NMR spectra 1H (Figure 11) and 13C (Figure 12) were realized in the DMSO-d6. The mass spectrum obtained by HS-ESI-MS in positive mode shows a pseudomolecuary ion at $[M+H]^+$= 277.09 (exact mass m/q= 276.09) corresponding to the raw formula $C_{11}H_{16}O_8$. The IR spectrum has an absorption band characteristic of the alcohol function at υ_{OH} = 3392cm^{-1}, the carbonyl function at $\upsilon_{C=O}$ = 1751 cm^{-1} and a double bond at $\upsilon_{C=C}$ = 1683 cm^{-1}. The 1H and 13C NMR characteristics are shown in Table 4. On the COSY spectrum, correlations are observed between protons H-6' and H-5', H-5' and H-4', H-4' and H-3', H-3'and H-2'. On the HSQC / or HMBC spectrum, we observe the correlations between the proton H-1' and the carbons C-4', C-3' and C-2' and the proton H-6' and the carbons C-5', C-4'. On the 1H NMR spectrum, there is a singular integrator for three protons at δ_H = 1.98ppm corresponding to the proton H-6. A singular integrating for two protons at δ_H = 4.72ppm corresponding to the proton H-5. Signals suggesting a hexose characterized by a doublet at δ_H=5.03(J=7.78 Hz) ppm corresponding to the proton of the anomeric carbon H-1', a doublet split at δ_H = 3.66 (ddd, J = 11.8/5.5/ 1.9 Hz) corresponding to a proton H-6', a triplet doublet at δ_H = 3.44 (dt, J = 11.8, 5.9 Hz) corresponding to the second proton H-6'and multiplets at δ_H = 3.23-3.15 ppm, δ_H = 3.14-3.08 ppm corresponding to protons H-5', H-2', H-4' and H-3'. The analysis of the Cosy spectrum and the mesuse of the coupling constant between the protons H-1' and H-2' makes it possible to identify hexose as the β-D-glucopyranose.[25] The 13C NMR and DEPT 135 spectrum shows in addition to the 6 carbons of sugar, the presence of five carbons including a primary (CH_3) at δ_C = 10.35ppm, a secondary (CH_2) at δ_C = 69.71ppm and 3 quaternaries at δ_C = 168.36, 137.96 and 136.65 ppm corresponding respectively to a carbonyl and two ethylenics. The correlations observed on the HMBC spectrum between proton H-2 and carbons C-4, C-3 and C-2 and proton H-6 and carbons C-5, C-4, C-3 and C-2 identified aglycone as 4-methyl-2(5H)-furanone. The junction between sugar and aglycone is justified by the HMBC correlation between proton H-1' and carbon C-3. All spectral data (IR, NMR and mass) in comparison with the data in the literature[25] confirmed the structure of the AJPP4 molecule as O-β-D-glucopyranosyloxy -4-methyl-2 (5 H)-furanone (figure 10).

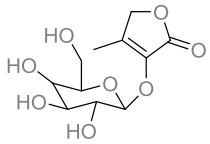

Figure 10 : Structure of the molecule of O-β-D-glucopyranosyloxy

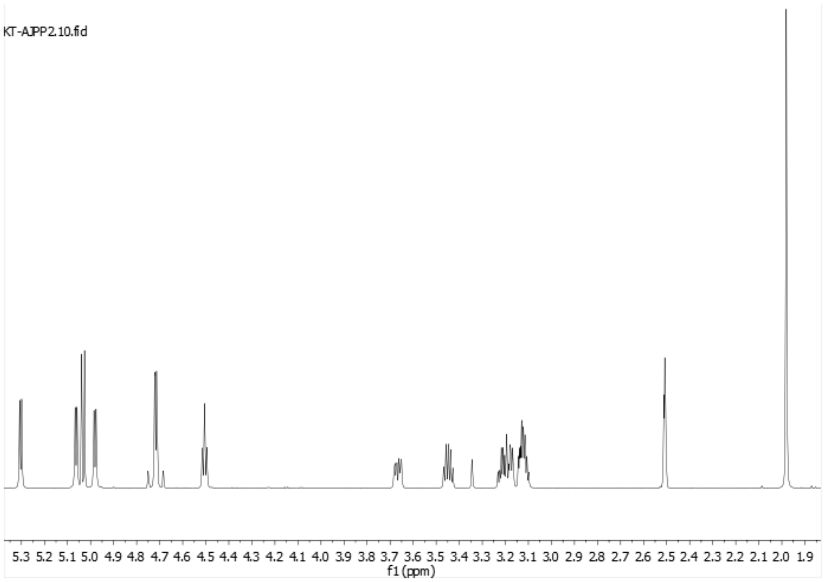

Figure 11 : 1H NMR spectrum of O-β-D-glucopyranosyloxy-4-methyl-2 (5 H)-furanone

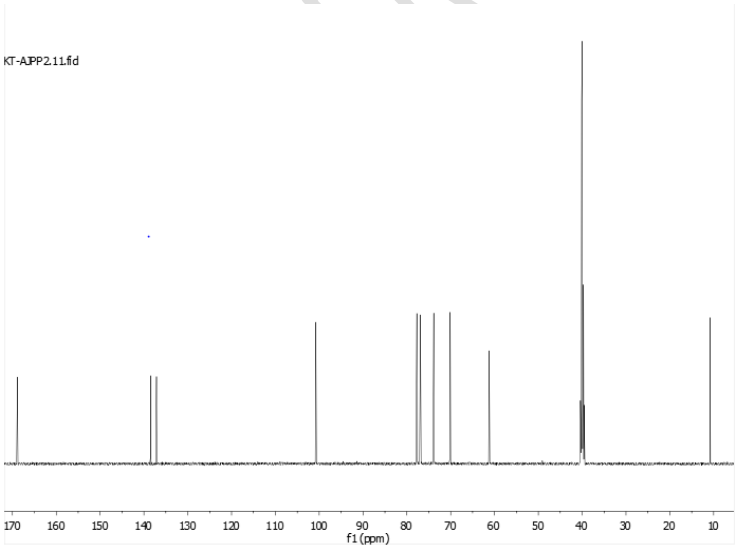

Figure 12 : 13C NMR spectrum of O-β-D-glucopyranosyloxy-4-methyl-2 (5 H)-furanone

Table 4: 1H and 13C NMR spectrum (in DMSO) of AJPP4

Position	13C NMR δ (ppm), J to Hz	1H NMR δ (ppm), J to Hz
C-2	168.36	–
C-3	136.65	–
C-4	137.96	–
C-5	69.68	4.72 (s)
C-1’	100.29	5.03 (d, J=7.78 Hz)
C-2’	73.39	3.15 – 3.23 (m)
C-5’	77.26	
C-3’	76.42	3.08 – 3.14(m)
C-4’	69.63	
C’-6’	60.73	3.66 (ddd, J = 11.8; 5.5; 1.9Hz)
CH_3-4’	10.35	3.44 (dt, J = 11.8; 5.9 Hz)

These 4 compounds, epicatechin, proanthocyanidin A-2, epicatechin-(2b→O→7, 4b→8)-epicatechin-(4b→8)-epicatechin (cinnamtannin B-1) and 3-O-β-D-glucopyranosyloxy-4-methyl-2(5H)-furanone are isolated for the first time in the extract of the roots of paullinia pinnata in Côte d'Ivoire. Epicatechin has various benefits for human health. This, due to its antioxidant, anti-inflammatory, antimicrobial, antitumor and cardioprotective properties.[26] Similarly, proanthocyanidins (oligomers of flavan-3-ol) are also of great interest in medicine and nutrition because of their antioxidant power and possible protective effects on human health.[27] Indeed, it has been shown that, highly polymeric proanthocyanidins possess healthy biological effects, including antioxidant effects[28], antimicrobial, antitumor, cardioprotective[27] antidiabetic[29-30] and anti-obesity.[31] This could justify the use of Paullinia pinnata roots in medicine against cardiovascular disease. Identification of the glycosil compound AJPP4 shows that this plant could be toxic.[32] However, well-monitored drying would eliminate this compound, which could lead to a reduced risk of toxicity.[33]

Determination of antioxidant activity

The antioxidant properties of the extracts obtained by conventional extraction with hexane (EHPP), dichloromethane (EDPP), ethyl acetate (EAEPP), methanol (MEOHPP) and and aqueous extract (AqPP), Ascorbic acid and three compounds (AJPP2, AJPP3 and AJPP4) isolated from the roots of *Paullinia pinnata* were also evaluated (Figure 13). The evaluation of antioxidant activity was carried out by the ABTS test (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid). The results are expressed in µmol T.Eq. (Trolox Equivalent) per gram of dry extract (µmol T.Eq./g extract). These results are reported in table 5.

Table 5 : Antioxidant activity of the extracts and the molecules of *Paullinia pinnata*

Extracts	Antioxidant activity [µmol Eq.T.] (µmol Equivalent Trolox per gram of dry extract)
EHPP	60
Aq pp	247
EDPP	316
EAEPP	372
AJPP2	556
MeOH PP	802
AJPP4	1263
ACIDE ASC	6901
AJPP3	10850

Comment [TS7]: How many times experiment repeated for confirmation of these values? Statistical expression should be made such as Mean with SD or Mean and SEM

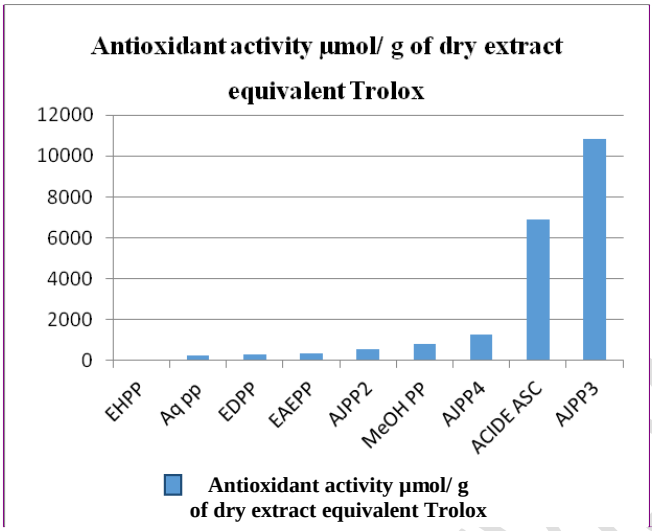

Figure 13 : Diagram of the antioxidant activity of the different extracts and the molecule of *Paullinia pinnata*

These results showed that all extracts and isolated molecules exhibit antioxidant power. The molecule AJPP3 (cinnamtannin B-1) has a better antioxidant activity compared to those of other extracts and that of ascorbic acid.

4-CONCLUSION

Paullinia pinnata is a medicinal plant with many therapeutic virtues. Phytochemical analysis of ethyl and aqueous acetate extracts of the roots of *Paullinia pinnata* made it possible to isolate and identify four (4) compounds, epicatechin, two proantocyanidins: proanthocyanidin A-2, epicatechin-(2b→O→7, 4b→8)-epicatechin-(4b→8)-epicatechin (cinnamtannin B-1) and a glycosil compound 3-O-β-D-glucopyranosyloxy-4-methyl-2(5H)-furanone. The evaluation of antioxidant potential showed that the different extracts and the three isolated molecules have antioxidant potential. The molecule of cinnamtannin B-1 (AJPP3) has a higher antioxidant power than ascorbic acid. These compounds are for the first time isolated from the species *Paullinia pinnata* from Côte d'Ivoire. The complete attribution of the four structures was established from spectral data IR, NMR (1H and 13C) and mass spectrometry.

Comment [TS8]: Number of times repeated for consistency of these tests results shall be mentioned in result part

REFERENCES

1 World Health Organization; Cardiovascular diseases, 2022.

2 M. Eddouks, M. L. Ouahidi., O. Farid, A. Moufid, A. Khalidi and A. Lemhadri, The use of medicinal plants in the treatment of diabetes in Morocco, Phytotherapy, 5(4), 194–203(2007)

3 World Health Organization. Cardiovascular Diseases, Promoting the Role of Traditional Medicine in Health Systems: Strategy for the African Region (Document AFR/RC50/9), Burkina Faso, 28 August-02 September 2000

4 P. Iserin, F. Moulard, R. Rachel, M. Biaujeaud, J. Ringuet, J. Bloch, E. Ybert, P. Vican, M. Masson, F. Moulard, J-P. Restellini And A. Botrel 2001, Larousse: encyclopedia of medicinal plants (2 ed), identification, preparation, care, Paris, pp.155-291

5 N. Bougandoura et N. Bendimerad, *Nature & Technologie, B-Sciences Agronomiques et Biologiques* ; n° 09/ Juin pp. 14(2013)

6 A. Zamblé, M. Carpentier, A. Kandoussi, S. Sahpaz, O. Petrault, T. Ouk and F. Martin-Nizard, *Journal of Cardiovascular Pharmacology*, **47(4)**, 599–608(2006) doi: 10.1097/01.fjc.0000211734.53798.1d

7 K. Annan, R. Govindarajan and E. Kisseih, *Pharmacognosy Journal*, **2(10)** 345-350(2010) https://doi.org/10.1016/S0975-3575(10)80107-5

8 J.D. Tamokou, J.R. Chouna, E. Fischer-Fodor, G. Chereches, O. Barbos, G.Damian, and R. Silaghi-Dumitrescu, *PLoS ONE*; **8(2)**, e55880 **2006**, https://doi.org/10.1371/journal.pone.005580

9 E.S. Iful, Studies On the Antivenom Activities of the Aqueous Extracts of *Paullinia Pinnata* and *Detarium Microcarpum* against Echis Carinatus (Carpet Viper) Venom. Ph.D. University of Jos. Pp.1-188 (2008), http://hdl.handle.net/123456789/214

10 I. M. Maje, J. A. Anuka, I. M. Hussaini, U. A. Katsayal, A. H. Yaro, M. G. Magaji, Y. Jamilu, M. Sani and Y. Musa, *Nig.Journ. Pharm. Sci*., **6 (2)**, 67-7(2007)

11 D. Strack, Plant *Biochemistry*, 387–416(1997), doi:10.1016/b978-012214674-9/50011-4

12 A.Latif, T.F. Khan and S.H. Afaq, *Pharmacologyonline*, **3**, 575-580(2006)

13 Lou, H.; A-type proanthocyanidins from peanut skins. *Phytochemistry*, **51(2)**, 297–308(1999), doi:10.1016/s0031-9422(98)00736-5

14 J. Lokvam, P.D. Coley and T.A. Kursar, *Phytochemistry*, **65(3)**, 351–358(2003), doi:10.1016/j.phytochem.11.012

15 A.M. Balde, L.A. Pieters, A. Gergely, H. Kolodziej, M. Claeys and A. J. Vlietinck, *Phytochemistry*, **30 (1)**, 337-342(1991), https://doi.org/10.1016/0031-9422(91)84150-Q

16 L.Y. Foo, J.J. Karchesy, *Phytochemistry*, **28(11)**, 3185–3190(1990), doi:10.1016/0031-9422(89)80303-6

17 A. Cronjé, J.F.W. Burger, E.V. Brandt, H. Kolodziej and D. Ferreira, *Tetrahedron Letters*, **31(26)**, 3789–3792(1990), doi:10.1016/s0040-4039(00)97472-0.

18 C. Santos-Buelga, H. Kolodzieij, D. Treutter, *Phytochemistry*, **38**, 499-504(1994) https://doi.org/10.1016/0031-9422(94)00637-9

19 V.A.P. De Freitas, Y. Glories, G. Bourgoisand C. Vitry, *Phytochemistry*; **49 (5)**, 1435-1441(1998), https://doi.org/10.1016/S0031-9422(98)00107-1

20 E. Le Roux, T. Doco, P. Sarni-Manchado, Y. Lozano and V. Cheynier *Phytochemistry*, **48 (7)**, 1251-1258(1998), https://doi.org/10.1016/S0031-9422(97)01070-4

21 N. Vivas, Y. Glories, I. Pianet, B. Barbe, an M. Laguerre, *Tetrahedron Letters*, **37(12)**, 2015-2018(1996), doi:10.1016/0040-4039(96)00187-6

22 K. Kamiya, C. Watanabé, H. Endang, S. Toshiko , *Chem. Pharm. Bull.*, **49(5)** 551-557(2001), https://doi.org/10.1248/cpb.49.551

23 N. Gen-ichiro, M. Satoshi and L. Nishioka, j. *Chem. Soc. perkin trans.*, 2139-2145(1983), https://doi.org/10.1039/P19830002139

24 J. C. Braekmant, D. Daloze and J. M. Pasteels, *Biochemical Systematics and Ecology*; 10(4), 355-364(1982), https://doi.org/10.1016/0305-1978(82)90010-2

25 M. Prakash, B.V. Basavaraj, and M.K.N. Chidambara, *Journal of Functional Foods*, **52**, 14-24(2019), doi:10.1016/j.jff.2018.10.021

26 D. Yu, T. Huang, B. Tian, J. Zhan, *Foods*, **9**, 1774(2020), doi:10.3390/foods9121774

27 K. Yokota, H. Kimura, S. Ogawa and T. Akihiro, *Journal of Chemistry*, *1–7(2013)*, doi:10.1155/2013/352042

28 S. Ogawa, H. Kimura, A. Niimi, T. Kastube, M. Jisaka and K. Yokota, *J. Agric. Food Chem.*, **56(24)**, 12046–12051(2008), https://doi.org/10.1021/jf802506r

29 M. Taher, isolation and in vitro antidiabetic properties of a proanthocyanidin from *Cinnamomumzeylanicum*; Thèse soutenue en **2005**

30 T. Yokozawa, E.J. Cho, C.H. Park. and J.H. Kim, *Evidence-Based Complementary and Alternative Medicine*, 1–11(2012), https://doi.org/10.1155/2012/623879

31 H. Kimura, S. Ogawa, A. Sugiyama, M. Jisaka and K. Yokota, *Food Res. Int*., **44(1)**, 121-126(2011), https://doi.org/10.1016/j.foodres.2010.10.052

32 https://en.wikipedia.org/wiki/Protoanemonin; consulté le **16/11 /2022**

33 http://www.horsedvm.com/poisonous/buttercup/; consulté le **16/11/2022**