CISSUS DINKLAGEI LIANA TANNIN EXTRACT CHARACTERIZATION

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ABSTRACT

The Cissus Dinklagei liana tannin extract was characterized using spectroscopy and spectrometry methods. The analysis of the ATR-FT, ^{13}C NMR and MALDI-TOF MS shown that this tannin is a condensed one and particularly a procyanidin type. It is mainly composed of chalcone, catechingallate, apidenin, gallocatechin, fisetinidin and quercetin. It has a very high percentage (54%**)**. This procyanidin types can be used as woods adhesives. The chalcon is a major constituent of this tannin. The presence of chalcone, gallocatechin, fisetinidin and quercetin may provide this tannin with a good antioxidant and anticancer properties.

Keywords: Cissus Dinklagei, Condensed tannin, characterization, Maldi-Tof MS.

INTRODUCTION

In the past, adhesives were manufactured using animal or vegetable substances, including egg white and animal blood (Pizzi and Mittal, 2003). The first phenol-formaldehyde resin adhesive was made in 1877, followed by adhesives based on urea-formaldehyde resin and urea molecule by Wölher (Mansouri, 2007). Nevertheless, the environmental problems resulting from the use of petrochemical adhesives and the first oil shock of the 1970s have led research in recent years to developed eco-friendly adhesives (Navarrete, 2011). The use of green resins in wood industry is not new (Pizzi, 1994), most cases of green adhesives were made with natural polyphenols including tannins. The condensed tannins or proanthocyanidols result from the polymerization of elemental molecules of flavans (flavans ol-3, flavane ol-4, flavanediol -3, 4) were particularly used because their reactivity with formaldehyde were high (Rahim and al., 2007). Since the 1970S, the use of condensed tannin as adhesives has already been established in some country. Many species, tree or tannins were used in the development of adhesives, such as mimosa, quebracho, aningre etc…..
In order to know more about the structure of tannins, many research works have been conducted. FT IR, 13C RMN, MALDI TOF methods have been used to analyze tannins (KONAI et al. 2015).

The lianas are exploited in various domains in Cameroon. The Waste from their exploitation is abandoned in nature. It is found in many country of Africa such as Cameroon, Democratic Republic of Congo (DRC), Gabon and Angola. In Africa, their leaves are eaten and stems sap are used for drinking. The fibers extracted from the stems are used as a bath sponge or as a rope. In Cameroon, it is found in abundance in all forests with a strong predominance in the

center and south of the country. Several pharmacological effects are recognized: antispasmodic, anti-inflammatory and anti-carcinogenic (Descoings 1972, Bosch 2004).
In view of having a sound knowledge of the chemical structure and in order to used it as adhesives, the structure of Cissus Dinklagei liana was extracted and characterized in this work using Fourier transform infrared, matrix assisted laser desorption ionization time of flight mass spectrometry and carbon 13 Nuclear magnetic resonance methods .

MATERIALS AND METHODS

CISSUS DINKLAGEI TANNIN EXTRACTION

250 g of Cissus Dinklagei ground bark was introduced in a water solution containing 2% sodium bisulfite and 0.5% sodium bicarbonate (water:bark ratio was 6:1).The whole was put in a bath water, under continuous stirring at 70°C during 4h. Then, proceeded to separation and filtration to obtain a reddish blackl iquid and a solid residue. The substrate obtained was concentrated at 60°C using a HEIDOLPH brand rotary evaporator, then it was frozen using liquid nitrogen and laboratory spray drier (KONAI et al., 2015).

CHARACTERIZATION

1. TOTAL PHENOL CONTENT

The total phenols content was determined by the Folin-Ciocalteu colorimetric method described by Scalbert and al., (1989) with a slight modification. The extract tannin was diluted in methanol to obtain a final absorbance between 0 and 0.5 (Saha Tchinda et al., 2013). 2.5 ml of Folin-Ciocalteu reagent (diluted ten times) was added to 0.0125 g of the tannin extract that was first dissolved in 25 ml of methanol. Finally, a solution of 7.5% of sodium carbonate was added to the mixture after two minutes. The reaction mixture was then stirred and incubated for 5 minutes at 50 ° C in a water bath. After this reaction time, the test tube was cooled and then the mixture was centrifuged with the SELECTA brand centrifuge at 500 revolutions for 15 minutes. The white of the solution was prepared by mixing 4.5 ml of distilled water with 0.5 ml of methanol. The absorbance was then read at 760 nm using the UV-visible spectrometer (UV-2550, brand LOVIBOND). A standard range (6 concentrations points between 0 to 25 mg / L) was made with gallic acid as standard of polyphenol. Through the standard range, the average concentration of the polyphenols present in the extractives was calculated as mg equivalents of gallic acid / g extractive which have been chosen as a control. **Fig. 1** show the calibration curve obtained.

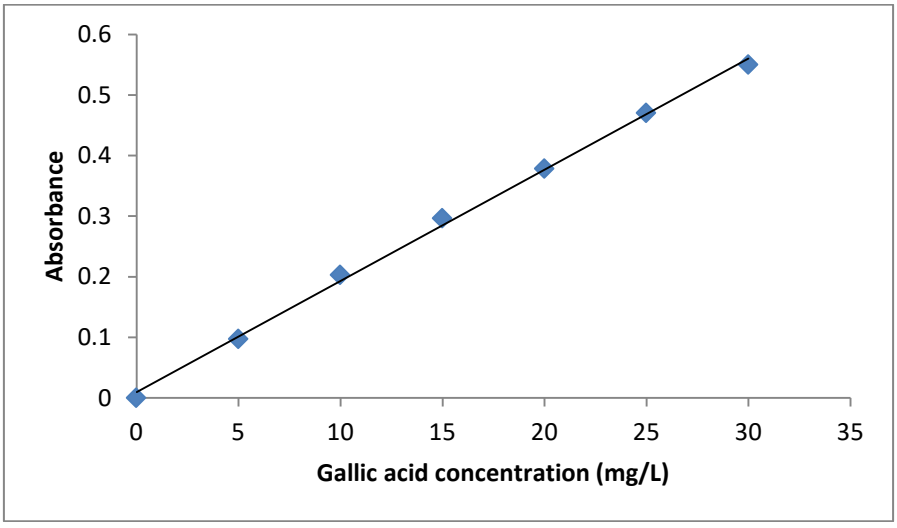

Fig. 1. Calibration curve: absorbance at 760 nm of different concentrations of Gallic acid

2. HYDROLYSABLE TANNIN CONTENT

100 mg of the tannin extract was mixed into a test tube containing 10 ml of distilled water, 2 ml of 37% formaldehyde and 1 ml of 10 M hydrochloric acid solution. Then, the solution was stirred and put in a water bath heated at 100 ° C during 30 minutes. At the end of the reaction, the solution is filtered under vacuum using a filter paper (No. 3). The solid residue was dried in an oven at 103 ° C during 48 hours. The rate of hydrolysable tannin (RHT) was calculated by the following equation (Ping and al, 2011):

$$RHT = \frac{m_1 - m_2}{m_1} \times 100 \qquad (1)$$

Where, m_1 is the mass of tannin and m_2 is the mass of extract after reaction.

3. CONDENSED TANNIN CONTENT

After obtaining the tannin extract, the content of condensed tannin was evaluated (Chamorro et al., 2012 and Saha Tchinda, 2015). A solution of 0.07% (w/v) of $FeSO_4.7H_2O$ dissolved in butanol / hydrochloric acid 950/50 (v / v) was prepared. 7ml of the prepared solution and 0.05g of tannin were introduced in a test tube. The test tube was immersed in a water bath heated at 95 ° C for 50 minutes, and carefully stirred to dissolve the extract. The tube is then cooled, and the absorbance of the solution is read at 550 nm, using a UV-visible spectrometer (UV-2550, brand LOVIBOND). The white of the reaction is obtained under the same conditions without the addition of the extract. The condensed tannins are expressed as cyanidin-3-O-glucoside equivalents, after making a standard range of the solution of this compound between 0 and 200 mg / l. **Fig. 2** shows the calibration curve.

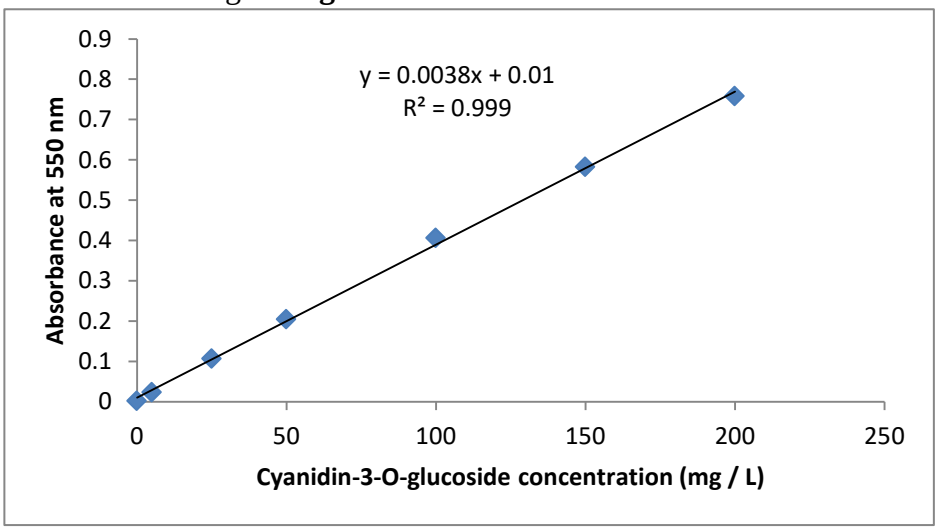

Fig. 2. Calibration curve: Absorbance at 550 nm of different concentrations of cyanidin-3-O-glucoside

4. ATTENUATED TOTAL REFLECTANCE FOURIER TRANSFORM MIDDLE INFRARED (ATR-FT MIR) ANALYSIS

A mass of 2 mg of fine powder of tannin extract is introduced at the diamond crystal / ZnSe of the BRUKER IR spectrophotometer analyzer. A manual force of about 150N is exerted on the sample to ensure contact. The sample was scanned 5 times, and each spectrum is obtained after 32 scans with a resolution between 4000 and 600 cm^{-1}**(**KONAI et al., 2015**).**

5. MATRIX ASSISTED LASER DESORPTION IONIZATION TIME OF FLIGTH (MALDI-TOF MS) ANALYSIS

Two solutions of acetone were prepared. The first consists in dissolving the tannin extract in a solution of acetone in the proportion of (5 mg / ml). The second was to introduce the matrix

(2, 5-dihydroxybenzoic acid) in a solution of acetone in the proportion (10 mg / ml). Both solutions are mixed in a 50/50 ratio to form a mixture (A). NaCl is added subsequently to accelerate the formation of the ions. 0.5-1µl of the mixture (A) is taken and placed on a plate (around a spot). After evaporation of the solvent for a few minutes in the free area, the wafer is introduced into the spectrometer for treatment. The MALDI-TOF spectra were obtained using COMPACT MALDI AXIMA PERFORMANE TOF2 software.

6. SOLID STATE CARBON 13 NUCLEAR MAGNETIC RESONANCE (^{13}CNMR)

Tannin extract from Cissus Dinklagie was prepared in solid form: Cross-Polarization Andle Spinning Magic (CP-MAS). Carbon Nuclear Magnetic Resonance spectra (^{13}C NMR)are carried out using a Bruker 400MHz MSL spectrometer (BRUKER Biospin, Wissembourgfrace). Chemical changes were calculated relative to TMS. The spectra were recorded at a rotor rotation speed of 12 KHZ on a 4 mm double movement controlled by the Bruker probe. The spectrum was acquired with a recycled delay of 5s at 90 ° pulsation of 4.2µs with a contact time of 1ms. The transition number was 3000. The spectrum was started with the deletion of one side of the wire band.

RESULTS AND DISCUSSION

PHENOL AND TANNIN

The total phenol content of the Cissus Dinklagei liana determinated using the Folin-Ciocalteu reagent is 84.88 ± 0.141 mg of gallic acid/g of extractives. The percentage yield of condensed tannin is 54.592 ± 0.05 g procyanidin eq / 100 g of dry matter and that of hydrolysable tannin is 0.272 ± 0.14 g. The total phenol contained in the liana is similar to those generally found in the lignocellulosic material. Elfalleh et al., 2012 during their study on pomegranate obtained the same result (85.60±4.87 mg gallic acid equivalents per g dry weight).The same observation was made in the case of condensed tannin relative to the hydrolyzable tannin (El falleh and al., 2012, BetuKasangana and al., 201; Saha Tchinda, 2015).

ATTENUATED TOTAL REFLECTANCE FOURIER TRANSFORM (ATR-FT) ANALYSIS

The analysis of the Cissus Dinklagei liana tannin ATR-FT spectrum **(Fig. 3)** revealed the presence of several functional groups specific to the condensed and hydrolysable tannins. The Peak 1751 cm^{-1} is associated to the C = O elongation vibrations of the esters group. The Peaks 1154 and 1204 $cm^{-1}$are associated to the aromatic C-H bending and stretching of aliphatic C-OH **(**Arianna and al., 2015). The Peaks 1245, 1440 and 1039 cm^{-1} are attributed to the combination of aromatic C-H flexion, C-O stretching and C-OH deformation. The deformation of A and B flavonoid nuclei is given by the peaks 767 cm-1. The Peak 1154 is attributed to the C-O bonds stretching and to the presence of gallic acid residues and carbohydrates in this tannin extract.The bands between 767 and 819 cm-1 represent the aromatic C-H bonds bending. The bands 1204, 1362 cm -1 peaks belonging to the aromatic nuclei (Socrate and al., 2000). The peak 1204 is specific to epicatechin and proanthocyanidin (Konail and al., 2017). The peak located at 1090 cm-1 is attributed to the C = O bond vibration of aromatic, esters and ketones. Peak 3324 cm^{1} represents ≡C-H binding of alcynes.

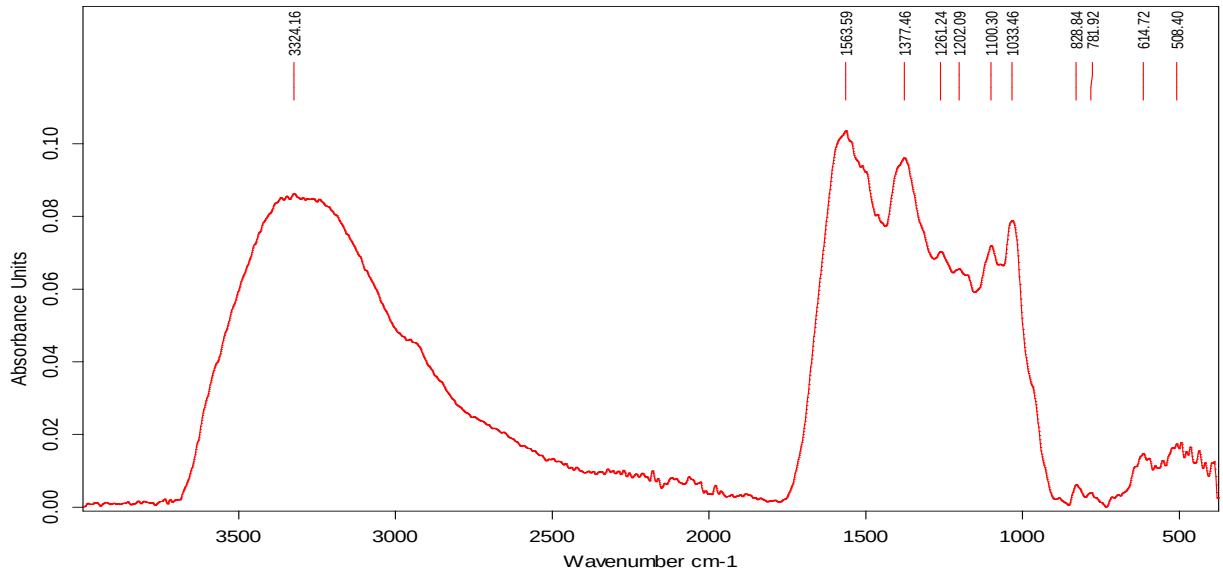

Fig. 3. IR-ATF spectra of *Cissus Dinklagei* tannin extract

MALDI-TOF ANALYSIS

The Analysis of MALDI-TOF spectra (**Fig.4** .a, b and c) in the range 200-1200 Da eight monomers present in this extract which combine with each other to form oligomers: catechin, gallocatechin, catechin gallate, fisetinidin, radicinin, chalcone, quercetin and apiginin. Their molecular masses are respectively: 290.3 Da, 306 Da, 442.4 Da, 274.3 Da, 236.22 Da, 208.25 , 302, 2 Da, 270.3 Da. They underwent modifications during their MALDI-TOF analysis, and either gained Na +, lost, or gained one or more hydrogen atoms (Saad and al., 2012). Some monomers or oligomers have also gained an OH molecule. The monomers or oligomers having gained or lost the hydrogen atoms with or without Na + are: 200 Da, 204Da, 316Da, 502Da, 523Da, 527Da, 559Da, 599Da, 605Da, 675Da, 701Da, 736Da, 764Da, 801Da, 805Da, 833Da, 838Da, 852Da, 866Da, 870Da, 881Da, 888Da, 929Da, 1012Da, 1142Da, 1159Da, 1176Da.Those who took an OH are (Navarret et al., 2010 and Drovou et al., 2015): 429Da, 457Da, 1058Da. It is important to note that the loss of H2O molecule is taken into account when forming certain monomers or oligomers; it's about: 551Da. Note also the presence of traces of sugar and galloyl. Peaks 456Da, 160Da show traces of galloyl. The presence of traces of sugar in the extract can be explained by the non purification of the latter, and is identifiable by the peaks: 178Da, 179Da. The most common monomers or oligomers are summarized in **Table 1**.

Table 1. Monomer and Oligomers structure of tannin from Cissus Dinklagei extract

Da	Relative Abundance (%)	Species	Remarks
160	30	Galloyloctaprotonated	Sugar residue
178	50	Glucose (-2H)	Sugar residue
179	38	glucose (-1H)	Sugar residue
200	100	Chalcone [-8H]	Flavonoid monomer
204	10	Chalcone [-4H]	
316	6	Quercetin +O	
429	4	Chalcone dimer +O	Flavonoid dimer
442	90	Catechin gallate	Flavonoid monomer
443	78	Catechin gallate protonated	
456	6	Catechin gallate +O	
457	6	Catechingallate +OH	
502	10	Chalcone + Catechin [-6H]	Flavonoid dimer
523	50	Chalcone + Catechinpentaprotonated + Na^+	
527	12	Chalcone + Quercetin[-2H]	
531	6	Chalcone protonated + Quercetin + Na^+	
551	30	Fisetinidin triprotonated + radicinin diprotonated+Na^+ [+HO]	
559	14	Catechin protonated + apigenin (-H)/Catechin +fisetinidin (-2H)	
561	6	Catechin +fisetinidin (-H)/Catechindiprotonated + apigenin	Flavonoid dimer
599	10	quercetin dimer (-3H)	
605	18	Gallocatechin +quercetin (-1H)	
627	2	Gallocatechine+quercetin +Na^+	
675	6	Catechin gallate + radicinin (-H)	
701	2	Catechin gallate protonated + radicinin diprotonated+Na^+	
736	2	Catechin gallate triprotonated + catechin triproprotonated	
764	2	Catechin gallate + quercetin protonated +Na^+	
801	16	Chalcone + Gallocatechin + catechin protonated	
805	26	Chalcones + Gallocatechin triprotonated + catechin deprotonated	
833	24	Chalcones triprotonated + Gallocatechin tetraprotonated + catechin tetraprotonated + Na^+	Flavonoid trimer
838	58	Fisetinidin dimer diprotonated + catechine	
852	76	Chalcones dimer + catechin gallate (-3H)	
866	64	Galloctaechin + catechin+ apeginine tetraprotonated	
870	50	Fisetinidin trimer heptaprotonated + Na^+	
881	44	Catechin gallate dimer (-H)	Flavonoid dimer
888.	100	Chalcon trimer + Apigenin (-2H)	Flavonoid tetramer
929	20	Gallocatechin trimer +Na^+ (-6H)	
960	12	Gallocatechin trimer + $2Na^+$	
1012	22	Catechingallate + gallocatechin + apigenin (-4H)	Flavonoid trimer
1058	12	Fisetinidine dimer + gallocatechin triprotonated (+OH)	
1142	20	Catechingallate dimer + radicinin diprotonated + Na^+	
1159	34	Catechin gallate dimer + fisetinidin tetraprotonated	
1176	54	Catechingallate dimer +catechin pentaprotonated	

This tannin extract, It is condensed tannin of procyanidin type. The presence of catechingallate, gallocatechin, catechin, fistinidin and quercetin gives it good properties for their uses as adhesives and may provide as a good antioxidant and anticancer properties.

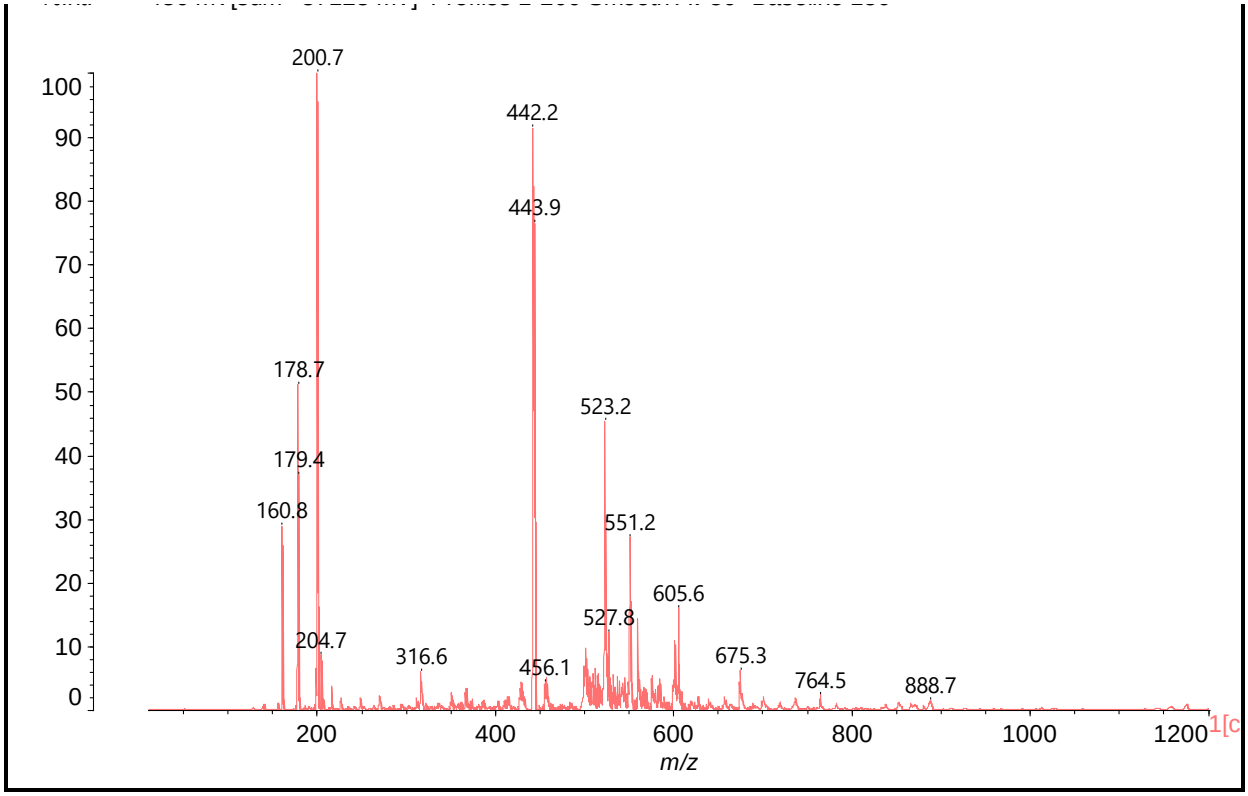

Fig 4.a. MALDI-TOF spectrum of the Cissus Dinklagei extract between 200-1200

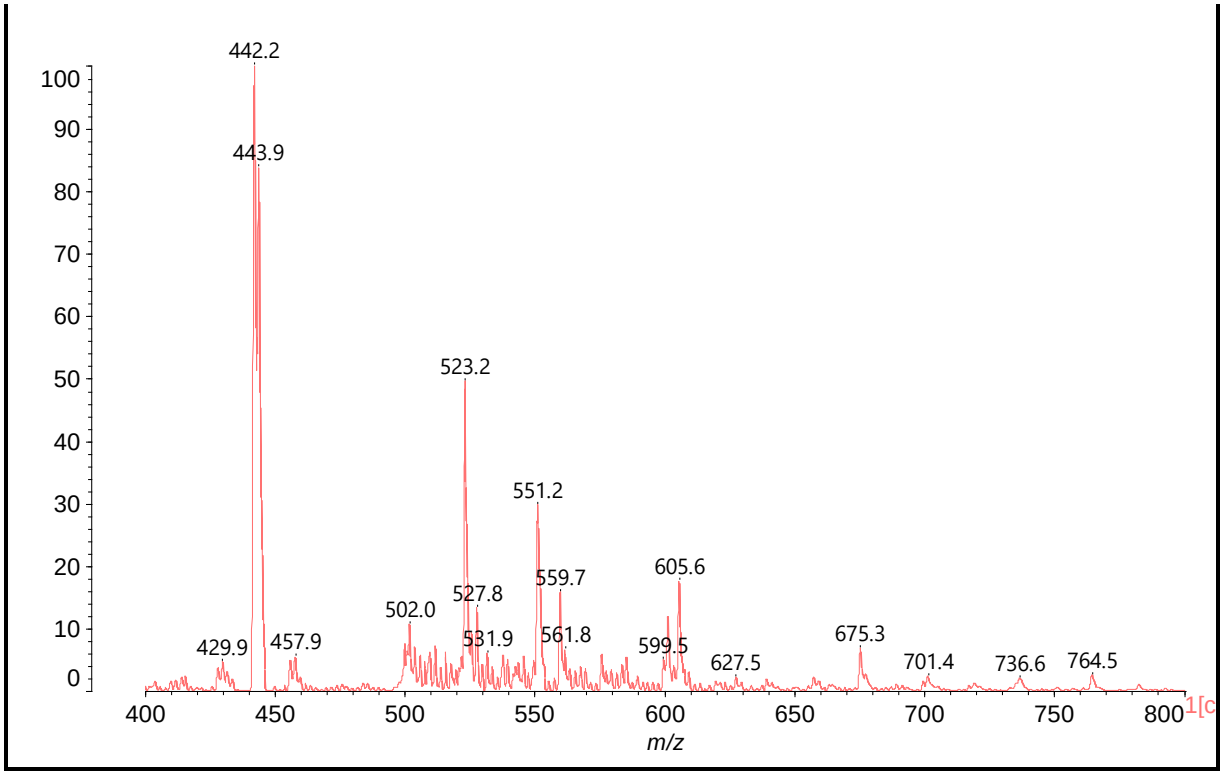

Fig 4.b. MALDI-TOF spectrum of Cissus Dinklagei extract between 400-800

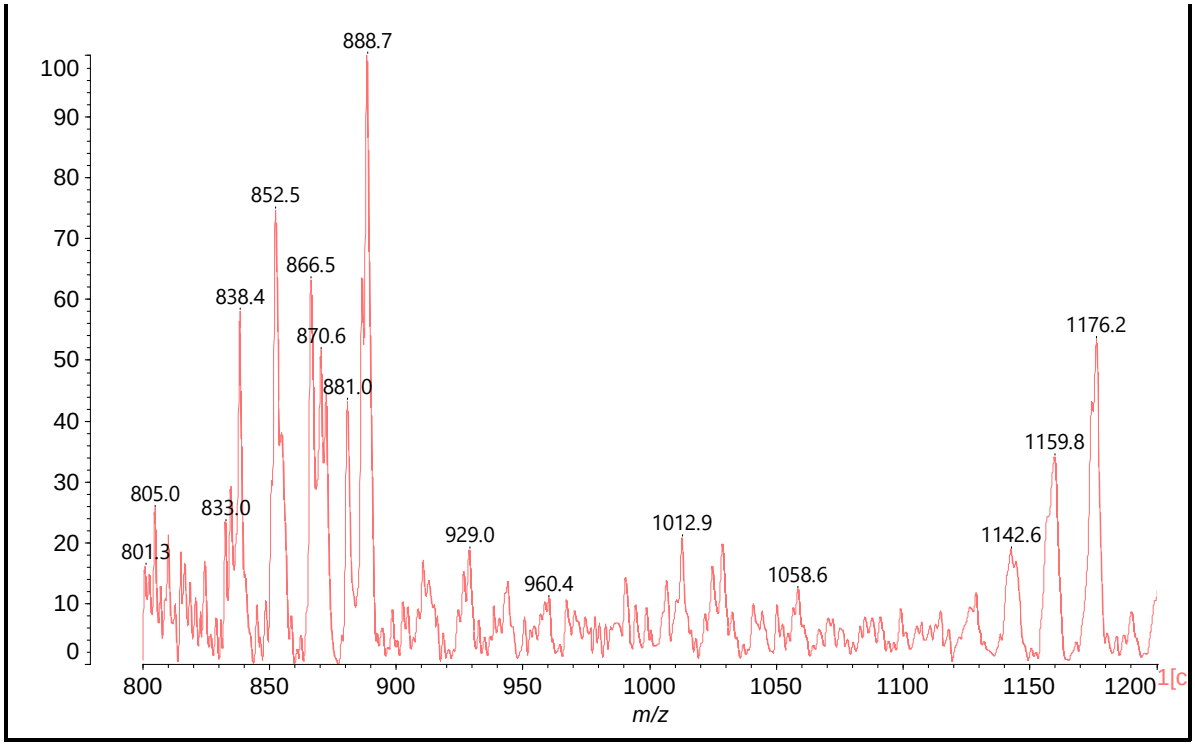

Fig 4.c. MALDI-TOF spectrum of *Cissus Dinklagei* extract between 800-1200

Structure of the monomers and oligomers present in the tannin of Cissus Dinklagei

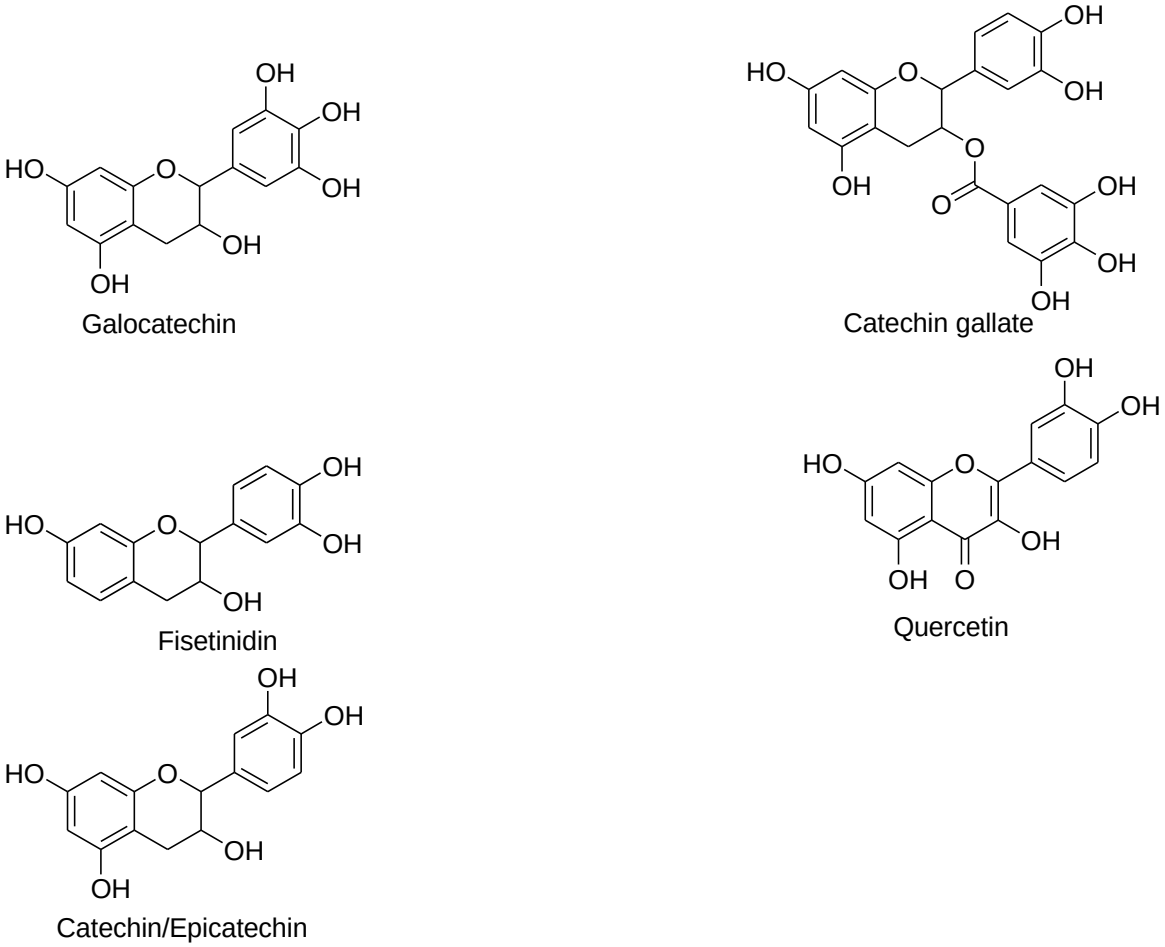

Fig 5. Structures of monomers and oligomers present in the tannin of *Cissus Dinklagei*

^{13}C NMR ANALYSIS

The examination of the 13C RMN spectra of Fig. 6 shows that, the peak 155.17 ppm of **(Fig. 6)** is attributed to the OH associated with the C5, C7 and C8 procyanidins. The peak around 144.41 ppm is associated with the C3 'and C4' ring B resonances of procyanidins. The band 131.23 ppm is attributed to C1 (Navarrete et al 2013). The latter may also be associated with C4'reflecting a small amount of prodelphinidin due to the overlap of procyanidin C1' chemical change (Ping et al 2011). Peak 107.03 ppm is assigned to C4-C8 inter-flavonoid bonds (Pizzi and Stephanou., 1993) but also to C6 'and C1' catechin. The peak around 98.42 ppm is associated with C6, C8 and C10, while C2 of the core C stereochemistry of procyanidins and carbohydrate residues is represented by the peak of 75.83ppm. The presence of the peak 71.26 ppm is assigned to the C3 of the B-ring flavonoids, OH flavonoids and traces of carbohydrates. Displacement of CH2 -O-CH2 bonds is represented by the peak 55.12 ppm. The latter not only reflects the presence of the methoxyl group (-OCH3) (Konaiet al., 2017) and the presence of glucose associated with flavonoids. The peak around 36.30ppm is associated with the C1, C5, C7 and C8 flavonoids and C4-C6 or C4-C8 flavonoid interlinks. In addition, the 31.73 ppm attributed to C3 is associated with an OH and C4 catechinic acid (Pizzi et al 1993). The peak signal 28.77 ppm is assigned to C4 catechin or C3 gallocatechin bearing hydroxyl groups.

Table 2. Summary of ^{13}C NMR analysis

Peaks (ppm)	Components or functional groupings
155.17	OH associated with C5, C7 and C8a procyanidins
144.41	C3 'and C4' Resonances of Procyanidin B Core (catechin / epicatechin)
131.23	C1 and C1 'procyanidins / C4' prodelphinidin
116.44	C5 'and C2' of B (procyanidins)
107.03	C4-C8 flavonoid interlinks / C6 'and C2' catechin
98.42	C6, C8 and C10
75.83	C2 C-core stereochemistry of procyanidins (epicatechin / epigallo-catechin), carbohydrate residues
71.26	C3 of the B core of flavonoids, OH of flavonoids and traces of carbohydrates
55.12	Carbohydrates and CH2-O-CH2 or the presence of the methoxyl group (OCH3) or flavonoid-associated glucoses
36.30	C1, C5, C7 and C8 flavonoids and flavonoid bonds C4-C6 or C4-C8
31.73	C3 associated with an OH, C4 Catechinacid
28.77	C4 catechin or C3 gallocatechine bearing hydroxyl groups

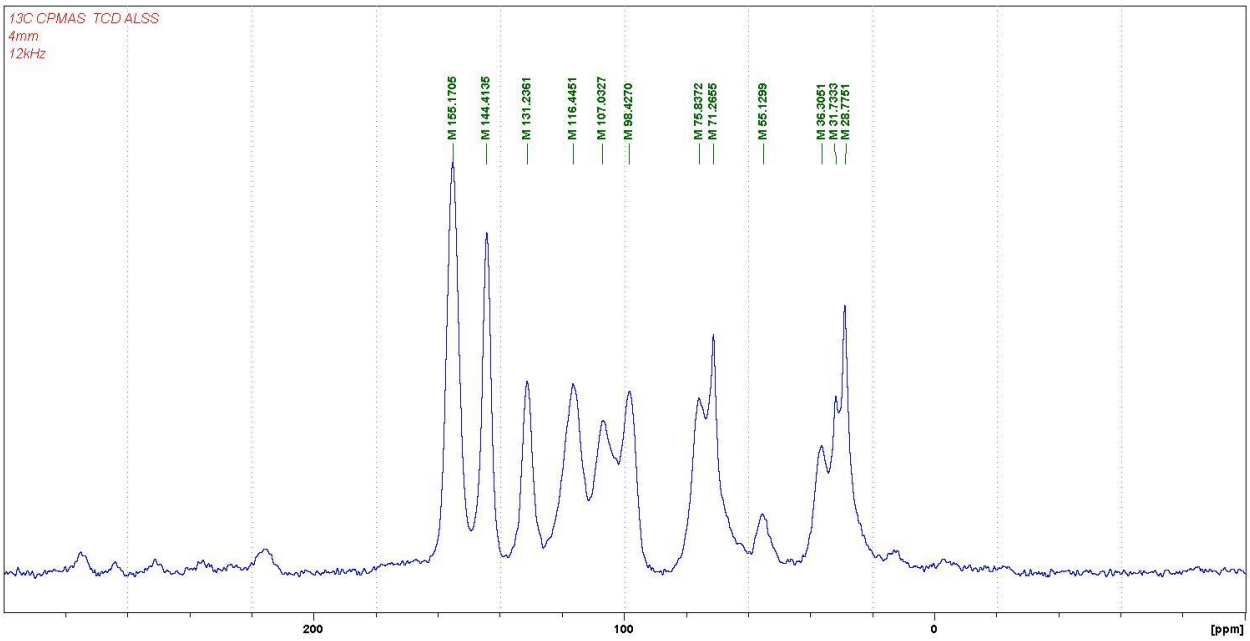

Fig 6. ^{13}C NMR spectrum of Cissus Dinklagei tannin extract

CONCLUSION

The Cissus Dinklagei tannin extract is a condennsed tannin. Its characterization using the following analysis techniques ATR-FT MIR, MALDI-TOF and 13C NMR, made it possible to pronounce on its structure and its typology. It is a procyanidine type composed of chalcon, catechingallate, gallocatechin, catechin, fisetinidin ,apigenin and quercetin, with a high yield of chlacone. The catechingallate, gallocatechin, catechin, fisetinidin and quercetin are known for their antioxidant powers, and also have very good properties for the manufacture of adhesives. This tannin can be used for the manufacture of particle board adhesives.

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