POTENTIALS OF *AZADIRACHTA INDICA* TANNIN EXTRACT

Noel KONAI[a,1], Antonio Pizzi[b], Karga Tapsia Lionel[c], Danwe Raidandi[a,c], MEVE'A Lucien[a] & Biyeme Florent[a].

[a]Laboratory of Civil Engineering and mechanical, University of Yaounde 1, BP 8390,Yaoundé, Cameroun
[b]ENSTIB – LERMAB, University of Lorraine, 27 rue du Merle Blanc, BP 1041, 88051 Epinal, France
[c]Laboratory of Materials, Architecture and Civil Engeniering, National Advanced School of Engineering, University of Maroua, BP 46, Maroua, Cameroun

ABSTRACT

The *azadiratcha indica* tannin extract is a flavonoid-polyethylene glycol complex. It is consisted of chrysin, myricetin, procyanidin catechin, gallocatechin, fisetinidin, chalcone, radicinin, catechin gallate, quercetin associated with ethylene glycol, diethylene glycol and triethylene glycol. It has adhesive, plasticizer and pharmaceutical properties. These properties were determinated using Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDI-TOF MS) methods. Used as adhesive, the Internal Bond strength of its particleboards based *Vachellia nilotica* exudate and paraformaldehyde as hardener were respectively 0,41 and 0,60 MPa. Their thermal behavior was identical; the difference appeared in the decomposition of the hardener used.

Keywords: *Azadiratcha indica* tannin. Properties. Plasticizer. Wood for adhesive. Pharmaceutical. Decomposition.

1. INTRODUCTION

The economy of several countries in the world and particularly that of some African countries is highly dependent on oil. Wood, plastics, textiles, automotive industries, etc… are highly dependent on it. Most of the composite materials manufactured in these industries are done with synthetics resins based on petrochemicals. The synthetics resins industries convert basic petrochemicals such as ethylene, vinyl chloride, propylene and styrene to manufacture a variety of resins or polymers. However, in recent years, the problem of environmental pollution has led researchers around the world to reduce dependence on petrochemicals. To replace at least 10% petrochemicals-based resins with biodegradable products or materials allows a substantial economic gain for society. Thus, several studies have been carried out using natural resources and particularly derivatives of plants (Konai et al., 2017; Pizzi, 2006; Ndiwe et al., 2019; Navarette et al., 2012). In a number of articles (Pizzi, 1978, Pizzi, 1994) wood resins using tannins have been developed.

It is for this reason that many African researchers have followed suit by working on bark tannins of different trees species (Konai et al., 2015; Drovou et al., 2015). The economy of several countries in the world and particularly that of some African countries is highly dependent on oil. The wood, plastics, textiles, automotive industries and other are highly dependent on it. Thus, to allow different countries to locally use tannins either in the wood or other industries, we extracted the *Azadirachta indica* bark tannin, a tree species strongly spread in Cameroon's Sahelian region and exploited in traditional African medicine as anti-inflammatory, antipyretic and antimicrobial (antibacterial, antiviral, antifungal, anticandidosis...) (Govindachari et al., 1998; Sithisarn et al., 2005).

[1] Corresponding author : noel.konai@yahoo.fr

To achieve the expected objectives, the *Azadirachta indica* (neem) bark tannin potentials were identified using MALDI TOF, ^{13}C NMR analysis, and used in the development of a bio composite. A thermogravimetric analysis was then carried out to control its behavior and degradation as a function of the temperature.

2. MATERIALS AND METHODS

2.1.*Azadirachta indica* tannin Extraction

In a bath water containing ground bark of *Azadirachta indica*, add a water (bark:water ratio was 1:6) and introduce a solution containing 0.4% sodium bicarbonate and 2% sodium bisulfite. Shake continusly the mixture at 60 ° C during 4 hours, filter the mixture and proceed to the concentration of the liquid obtained at 60 °C using a rotary evaporator. Then the liquid fraction obtained was frozen using liquid nitrogen and a laboratory spray dryer to obtain a tannin powder (sealy-fisher and Pizzi, 1992).

2.2.Extraction of hardeners from trees exudates

Using a small blade or machete, make an incision about 60 cm deep on the *azadirachta indica* tree trunk, and then remove the bark manually. After fifteen days of bleeding, the exudate is collected in a container, dried at ambient temperature during three weeks and crush it in the form of powder or crystals ready to use.

2.3 **Matrix Assisted Laser Desorption Ionisation Time Of Flight Mass Spectrometry (MALDI-TOF MS)**

5 mg of sample were dissolved in 1 mL of acetone. The sample solution was mixed with another one consisting of 2,5 - dihydroxybenzoic acid as matrix and an acetone solution (10 mg/ mL acetone). The ion formation was enhanced adding sodium chloride (NaCl) to the matrix (10 mg/mL in distilled water). The resulting solutions were evaporated on the MALDI target before placing into the spectrometer. The spectra were recorded on compact Axima Performance MALDI TOF 2 mass spectrometer (Shimadzu Biotech, Kratos Analytical Shimadzu Europe Ltd., Manchester, UK). The irradiation source was a pulsed nitrogen laser (wavelength: 337 nm, laser pulse length 3ns, and target type: ground steel) (Konai et al., 2015).

2.4 Solid state ^{13}C Nuclear Magnetic Resonance (^{13}C NMR)

Solid state Cross-Polarization Magic Angle Spinning (CP-MAS), ^{13}C Nuclear Magnetic Resonance (^{13}C NMR) spectra were recorded on a 400 MHz Bruker MSL spectrometer (Bruker Biospin, Wissembourg, France). Chemical shifts were calculated relative to tetramethylsilane (TMS). The rotor was spun at 12 KHz on a doublebearing 4 mn Bruker probe. The spectra were acquired with 5 s recycle delays, at 90° pulse of 4.2 µs and a contact time of 1 ms. The number of transients was 3000. The spectra were run with suppression of spinning sidebands (Konai et al., 2017).

2.3.adhesive development

Mix an aqueous solution containing 30% tannin + 5% hardener based weight and adjust the pH to 10 adding sodium hydroxide. Then stir the mixture until a viscosity equal to 600 cp. The viscosity was measured with a Brookfield RV viscometer.
Two hardener were tested: the *Vachellia nilotica* tree exudate and paraformaldehyde.

2.4.Internal Bond (IB) Strength.

The Internal Bond Strength of particleboards manufactured during 7 min 30 s pressing time divided in three steps (28kg/cm^2, 3min - 12kg/cm^2, 2- 5.8kg/cm^2, 2min) was determined

according the International standard NF EN 312-2 (1996) using INSTRON 4467 universal testing machine.

2.5.Thermogravimetric analysis (TGA)

About 100 mg of each of particles board was placed on a balance located in the furnace and heat was applied over the temperature range from 20 to 900°C at a heating rate of 5°C/min during 30 min in argon gas. This test was done using NETZSCH STA 449F3 Jupiter (Germany) equipment. The Weight gain (TG) curves and mass loss rate (DTG) were determined using excel software

3. RESULTS AND DISCUSSION

3.1. Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDI-TOF MS)

The MALDI TOF spectrum in Fig. 1 shows the presence of a flavonoid-polyethylene glycol complex consisted of chrysin (254 Da) ; myricetin (318 Da) ; catechin (290 Da), gallocatechin (306 Da) ; fisetinidin (273 Da) ; chalcon (270 Da) ; radicinin (236 Da) et catechin gallate (442 Da); quercetin (302.2 Da); ethylen glycol (62,07 Da) ; diethylenglycol (106,12 Da) triethylen glycol (150,173 Da) (Table 1). The molecular weights obtained take into consideration that of Na + (23 Da) contained in the NaCl used as matrix (Konai et al., 2015). In this MALDI TOF analysis, the protonated form and the loss of one or two hydrogen atoms and hydroxyl group must be taken into account if they are not the terminal units of oligomers (Saad et al., 21012). The complete list of the monomers present in this tannin extract is summarized in Table 1. The presence of a flavonoid-polyethylene glycol complex showed that this tannin extract has a plasticizer, in the pharmaceutical industry and as adhesive. It can be used as a plasticizer because ethylene glycol and diethylene glycol are usually used as plasticizers for plastic and trimethylen glycol is used as plasticizer for vinyl products. The existence of catechin which showed an interesting protective effect in people on dialysis confers to the extract the properties of a medecinal product. This extract may be a good antioxidant and had anticancer properties due to the presence of quercetin, catechin, fisetinidin, myricitin, gallocatechin, chrysin and myricetin (Albu et al., 2009). Regarding the spectrum, the quercetin, a powerful antioxidant, had a high percentage. Conversely, the chrysin exhibits many biological activities and pharmacological effects, including antioxidant, anti-inflammatory, anticancer, and antiviral activities. The presence of flavonoids such as catechin, gallocatechin, fisetinidin, chalcone, radicinin, catechin gallate, quercetin etc ... proved that this extract is a condensed tannin; and then, it can be used for wood adhesives (Pizzi, 1978). The new monomers and oligomers identified in this extract are shown in figure 2.

Table 1: Monomers present in the *azadiratcha indica* tannin extract

M (Da)	Oligomères
211/212	2 Diethylene glycol
238	2 Diethylene glycol (+OH)
256	Chrysin (+2H)
277	Chrysin+ Na^+
291	Catechin (+H)
318	Myricetin
320/321	Myricetin (+2H)

450/451	Tryethylene glycol+quercetin (-H)
466/467	Tryétylene glycol + myricetin
477	2 Chalcone+ethylene glycol
494/495	2 Radicinin + Na^+
520/521	Catechin+diethylene glycol +2 ethylene glycol (+2H)
561	2 Radicicinin+ Na^+ (+H)
563/564	Fisetinedin + catechin
620	Myricetin+quercetin
630	Gallocatechin+quercetin+ Na^+ (-H)
730	2 Catechin+ triethylene glycol
794	Catechin+ethylene glycol+ catechin gallate
820	2 Gallocatechin+chalcone
846	2 Myricetin+ chalcone

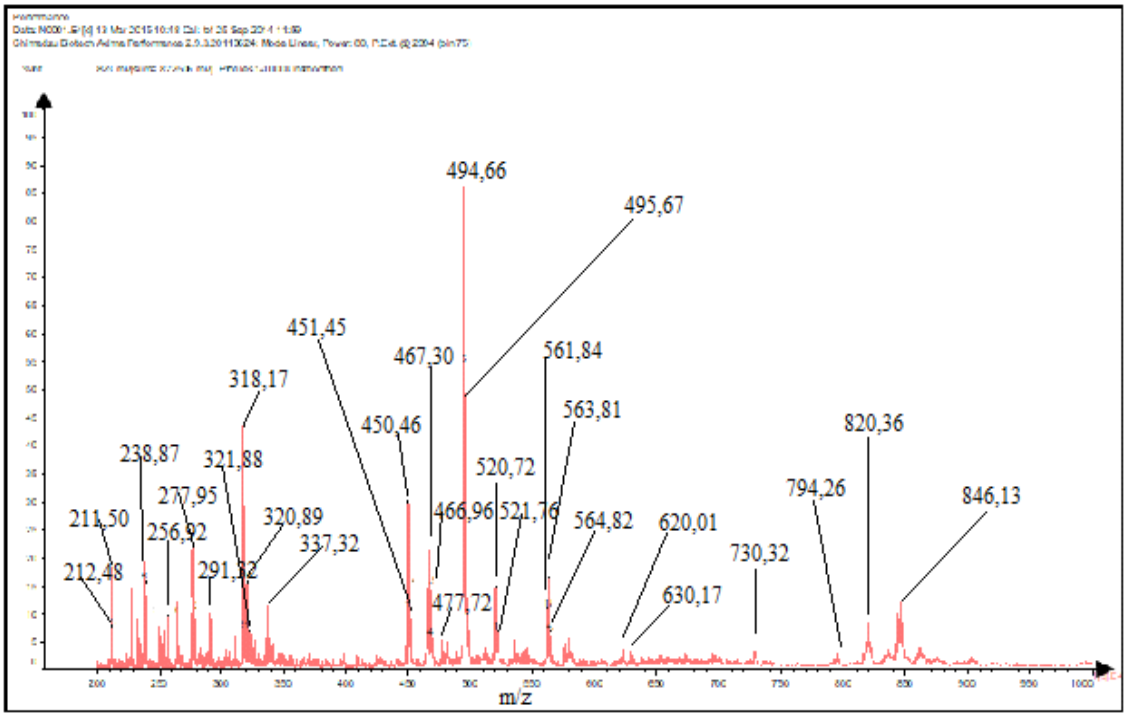

Fig.1: MALDI -TOF of *Azadirachta indica* tannin in the 200–1000 Da range

Table 1: Flavonoid-polyethylene glycol complex of *Azadirachta indica* tannin extract

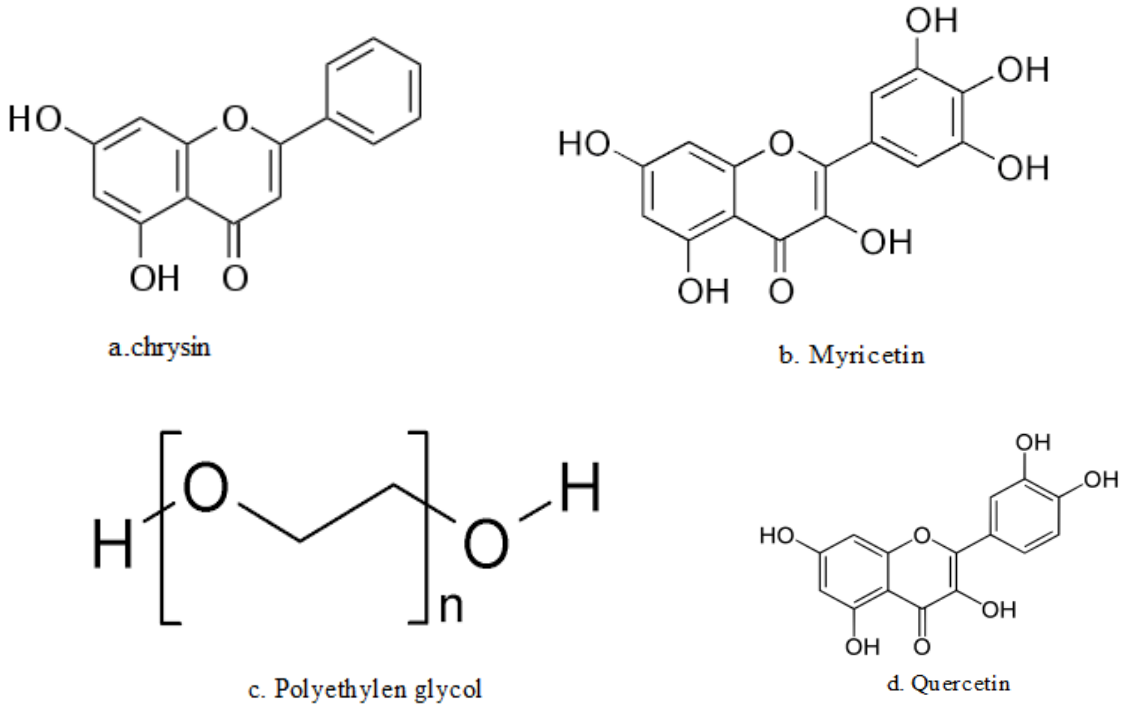

Fig. 2 : Monomers and oligomers structures presents in *azadirachta indica* tannin extract

3.3. ^{13}C RMN of the tannin extract of *Azadirachta indica*

The ^{13}C RMN spectrum (Fig. 3) analysis of the *Azadirachta indica* tannin extract showed the presence of various functional group. The presence of quinone structures due to the oxidation of phenolic hydroxyl group is traduced by the peak 193.01 ppm. The peaks 174.01 and 168,40 ppm ppm indicates the existence of C = O bonds associated to gallocatechin or catechin gallate,, fisetinidin, chalcone (Davis et al., 2010). The peak 153, 23 ppm represents the C5, C7, and C8a carbons of procyanidins (Zhang et al. 2010). The existence of a catechol B ring can be traduced by the presence of the peak 129, 39 ppm. The peak 103, 89 ppm is corresponding to C2′and C6′ of catechin. The shoulder located at 82, 35 ppm is sensitive to the stereochemistry of the C-ring. The ratio of the 2,3-cis to 2,3-trans isomers could be determined through the distinct differences in their respective C2 chemical shifts, it also gives a resonance around 74.83 ppm for the cis form and 82,35 ppm for the trans form. The C3 of both cis and trans isomers are located at 72,53 ppm (Zhang et al., 2010). The formation of $–CH_2–O–CH_2–$ bridges can be observed at 72, 53 ppm. The 62, 46 ppm belongs to C3 in the terminal unit. The peak at 30, 34 ppm belongs to free C4 sites of the flavonoid (Basso et al., 2014). The peaks at 55, 58; 33,14 and 23,32 ppm are attributed to the presence of carbohydrates residues in the *Azadirachta indica* tannin extract.

According to the ^{13}C RMN and MALDI-TOF MS analysis, the *Azadirachta indica* tannin is a condensed one and particularly a procyanidin type, usable as a plasticizer, adhesive and in medecine as antioxydant, anti-inflammatory, anticancer, and antiviral.

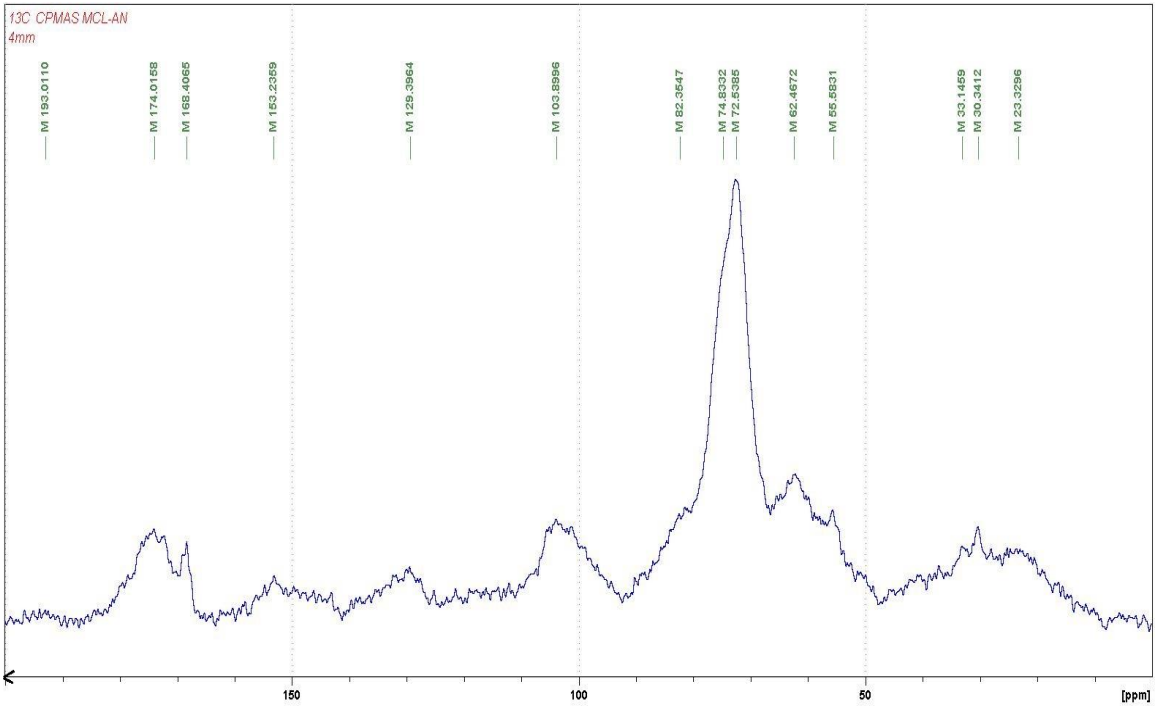

Fig. 3: ^{13}C NMR spectrum of *Azadirachta indica* tannin

3.4. Composite development and internal Bond strength

The Fig. 4 bellow indicates that the Internal Bond (IB) strength of particleboards bonded with the resin based on this tannin and 5% paraformaldehyde as hardener was higher than that using the exudate of *Vachellia nilotica* as biohardener (0.6> 0.41 MPa). However, that using 10% of *Vachellia nilotica* was higher than that of particleboards using 5% of paraformaldehyde. This can be explained by the much higher average molecular weight of the exudate in relation to the small molecular weight of formaldehyde. Thus, when the proportion of *Vachellia nilotica* exudate biohardener is double that of paraformaldehyde, the IB strength of the particleboards is higher than that based on paraformaldehyde.

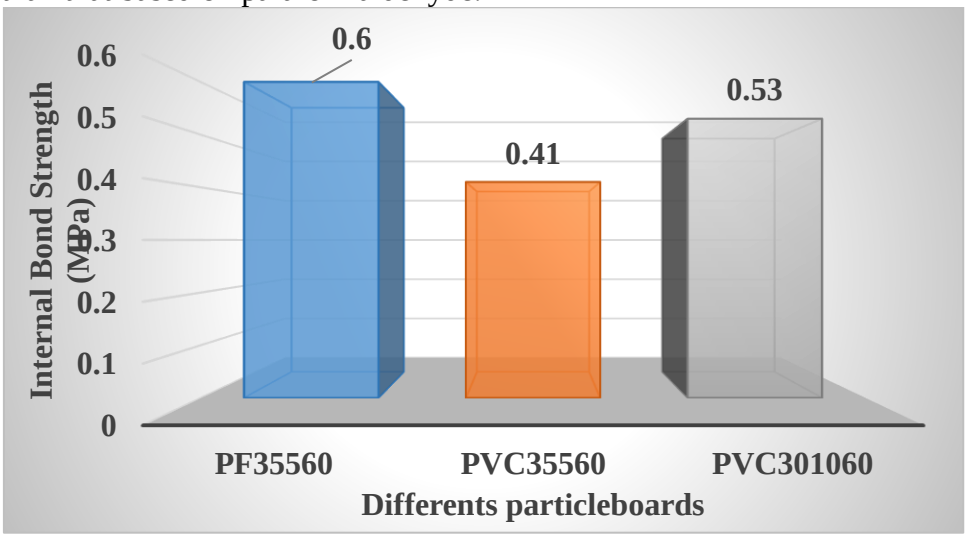

Fig. 4 : Internal Bond srength of various particleboards

PF35560 : Particleboards based resin containing 35% tannin + 5% paraformaldehyde as hardener + 60% water

PVC35560 : Particleboards based resin containing 35% tannin + 5% vachellia nilotica exudate as bio hardener + 60% water

PVC301060 : Particleboards based resin containing 35% tannin + 10% vachellia nilotica exudate as bio hardener + 60% water

3.6. THERMOGRAVIMÉTRIC ANALYSIS

The Figures 5 and 6 show the TG (TG_Comb) and DTG (DTG_ Comb) curves of PF35560 and PVC35560. These thermogrammes, shows three main regions of mass losses at different temperatures.

For PF35560 (Figure 5), the first mass losses region is between 40 and 150 ° C;
The peak at 56°C is mainly due to the loss of absorbed water and trace formation of volatile compounds such as CO, CO_2 and CH_4 released by the paraformaldehyde of the resin and the particleboards.

The second phase (transformation into gaseous products) takes place between 150 and 350 ° C, around 295 ° C occurs the degradation of the resin and particularly that of tannin and the degradation of paraformaldehyde releasing CO, CO_2 and hydrogen.

The third phase (complete combustion) occurs between 350 ° C and 900 ° C. the peaks located at 395, 425 and 439 ° C represent the release of gaseous products such as CO, CO_2 and CH_4; From 485 ° C, the particleboards which yielded a "gaseous product" during the previous incandescent phases, produce embers. A temperature of 500 ° C at least is sufficient to ensure complete combustion of the PF35560 and it is since this temperature is reached that the gases difficult to "burn" were burnt.

The three phases observed during the ATG of PF35560 are observed during the ATG of PVC35560. However, the analysis of fig.5 brings out some remarkable combustion points: 56 ° C; 275 ° C; 299 ° C; 425 ° C; 445 ° C; 459 ° C; 470 ° C. The peak around 56 ° C traduce the evaporation of volatile organic compounds. Those located at 275 and 299 ° C represents the degradation of the resin and particularly, that of the *Vachelia nilotica* exudate and in particular that of the wood particles. The peaks at 425°C; 445°C; 459°C et 470°C represent the combustion of rigid elements resulting in the release of gaseous products.

The differences observed between the temperatures of two ATGs (PF35560 and PVC35560) are explained by the degradation of the different hardeners used.
TG :Thermogravimetric
DTG : Derived of thermogravimetric curve

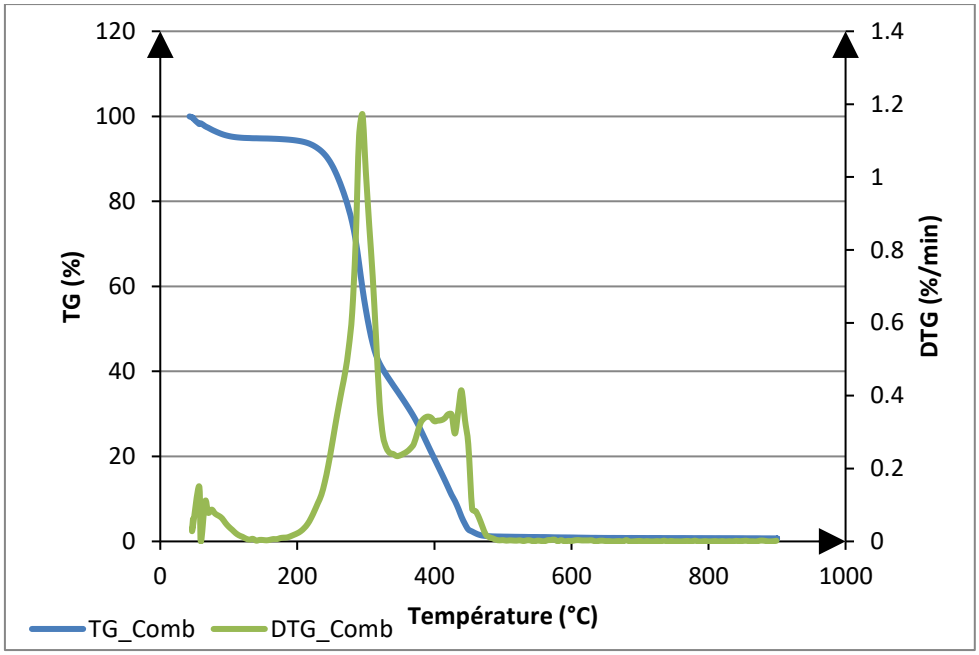

Fig.5: TG and DTG curves of PF35560

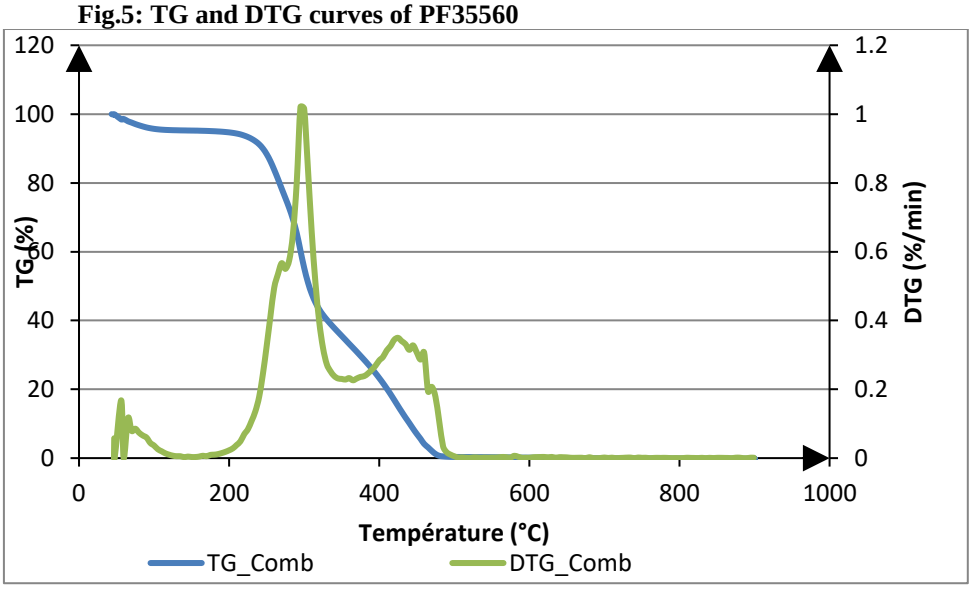

Fig. 6: TG and DTG curves of PVC35560

CONCLUSION

The *azadiratcha indica* tannin extract has adhesive, plasticizer and pharmaceutical properties. Its use as woods adhesives gives encouraging results. This tannin will be used as plasticizer in further paper.

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