"Extraction and phytochemical screening of tannins from the barks of ficus sicomorus, zizyphus mandermae and azadirachta indica in order to develop a natural binder for the manufacturing of a composite material"

DANWE RAIDANDI, NOEL KONAI, SAIDJO.

Abstract: **The tannins, polyphenolic components used by plants to defend themselves against insects and other attacks, present in the cytoplasm of plants and formerly used for tanning are today used as raw materials for the development of adhesives to manufacture composites materials.**

Three types of tannins have been studied in order to make a binder for the manufacture of a composite material: The extracts of the barks of Azadirachta Indica, Ficus Sycomorus and Zizyphus Mandermae from the woods species of Cameroonian flora.

Some phytochemical screening has permitted us to find out the presence of tannins (Gallic, ellagic, catechic) and some other very useful constituents for the manufacturing of adhesive namely: the *Flavonoids and carbohydrates.* Some sweet substances present in the extracts were removed during the extraction with acetone + ethyl acetate.

To extract those tannins we initially did an extraction with normal water then with acetone + acetate ethyl in order to eliminate the impurities.

After the development of a binder with each extract, we find out that between those three types of woods, only the tannin of Ficus Sicomorus allowed us to develop an adhesive with the best features: a quick-setting glue with a shorter gel time ranges from 50 and 152 seconds at $PH = 10$; stick metals, plastics and woods materials. Gel time decreases for $7 < pH < 11$ and increases for $PH > 11$.

The presence of flavonoids and carbohydrates help to improve the adhesive strength, the *carbohydrates increases the viscosity* of the binder.

Keywords: tannin, binder, flavonoïds, carbohydrates, composite materials.

I. INTRODUCTION

The Binders are used to link or to put together other materials. They are materials that have the property to solidify itself then become hard by acquiring mechanical characteristics (resistance in reduction, traction, adhesion).

Nowadays, most adhesives used in our industries, particularly in the wood industries are pollutants and are using petroleum resources and formaldehyde as cross-linking agents: This is the case with formalin Urea, Formaldehyde Phenol, Formaldehyde Melanin… thanks to the:

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- Classification of formaldehyde as a carcinogenic substance, polluting and harmful;
- environmental pollution against humanity;
- exhaustion of petroleum reserves, basic material still then used in the development of synthetic adhesive;
- search of making composites materials and rehabilitating local materials and particularly flora essence of Cameroon;
- Worrying to exploit the scraps (barks) used in Cameroon sawmills…

The creation of a natural binder within these last decades has become a major worry for the international scientific community.

The main objective of this work, is to characterize and extract tannins from the barks of the Ficus Sicomorus, Zizyphus Mandermae and Azadirachta Indica, to develop a binder that will be used to make a biodegradable material composed only with vegetal substances, to develop a binder to produce a biodegradable material and to obtain a gel time of less than 200 seconds.

In order to do this, we are going to draw a list of materials and methods used, then give the results and test it by doing the chemical identification of the molecules present in the tannins; extract the raw tannin using water then acetone +ethyl acetate to eliminate the sweet substance to obtain a pure tannin.

II. MATERIALS AND METHODS

The Barks (specimen) of three species (tale 1) of plants came from the Cameroonian flora particularly from the Far North Region of Cameroon, Mayo stanaga Division, Zidim village.

The barks are firstly dried in the sun for two (02) weeks then grind into particular inferior or equal to 5 mm of diameter; then put into packets of 150 grams each and finally labeled per species.

Before the extraction, the tests using iron chloride by stiansy's reactant (formalin at 30 %: 2 volumes; chlorydric acid: 1 volume), by ethanol chloride and magnesium permitted us to identify the presence of tannins. After extraction of the tannin to gross water, we washed with acetone + acetate ethyl to eliminate sweet substance and to obtain pure tannin.

The experiments were carried in the pharmaco chemistry Laboratory of Natural Substances of the University of Yaounde 1.

A. Phytochemical screening of tannins

To obtain the contents of tannins in percentage of dried barks, the calorimetric dose method with iron salt using a tampon of $PH = 8.3$ has been adopted [1].

The dosage of tannins from the barks of these three species named above permitted us to observe that they contain tannin in the following proportions.

An investigation done on the field (in far north region of Cameroon, Mayo Stanaga Division, Zidim village) permitted us to observe three different areas occupied by the different species.

B. Extraction

Extraction with normal water and acetone ethyl acetate

In a round – bottomed flask of 2 L, put 150 g of barks of the plants, then a watery solution which volume of water is equal to 6 times the volume of barks used are calculated from the dried volume. The solution is put in a bain – Marie heated to $80°C$ and shaken for 4 hours.

Then the separation and filtration are done with an empty Buchner. Use a Buchner funnel with a paper filter and an empty flash linked to an empty source. We obtain a black and reddish liquid and some hard remains. The liquid fraction obtained is then concentrated using a rotative evaporator at a temperature of $60\ °C$.

After evaporation, add acetone + ethyl acetate (50: 50) to eliminate the sweet substances and to

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obtain pure tannin and finally proceed the evaporation again for both (liquid and organic) phases obtained.

C. Characterization

Highlighted of tannin

To identify the presence of tannin, we have to:

- Extract the colourant from the bark: $5g$ of crushed bark + $100\,ml$ of boiling $H2O$ + 15 min of agitation and filtration;
- Add $1\,ml$ drops of $FeCl3$;
- Add $1\,g$ of sodium acetate;
- Observe a colour characteristic of tannin.

Test presence of flavonoid: WILSTATER'S test

In 5 ml of filtrate, add $5\,ml$ of $(C_2H_50H + HCL)$ + powder of magnesium. It will appears to be coloured followed (with effervescence). To this alcoholic solution, add some drops of iron chloride it will change to an orange colour **[2].** After 15 min, the presence of flavones is shown through the change into a red colour, when there are flavonols or flavanones we can see the change of the colour into crimson red or purple red.

The leucoanthocyanes: Bate-Smith test

In a round – bottomed flash containing $15\,g$ of barks and $0.5\,ml$ of condensed HCl $(12.07\,N)$ then heat the solution in a water bath at 80 ° C for 40 min. after cooling, a red colour indicating the presence of leucoanthocyanins **[1]**.

Highlighted hydrolysable tannin

With an aqueous solution of ferric chloride, the ellagic and gallic tannins give colours and precipitates **[3];** this reaction allows highlight phenolic acids.

6 **Reaction of differentiation**

- At $15\,ml$ of brewed add $8\,ml$ STIANSY reactant (formalin 30%: 2 volums of hydrochloric acid: 1 volume).

- Heat the resulting solution for 30 minutes at the boiling temperature. The formation of a precipitate shows the presence of condensed tannin.

- Filter, the filtrate using sodium acetate then some drops of iron chloride (2% added); it appears coloured indicating the presence of hydrolysable tannin precipitate by stiansy's reactant **[3]**. **Note**: we can characterize these tannins with other reactants: With potassium iodine, the gallic tannins give a pink precipitate, in the presence of nitrous acid in an acid environment, the ellagic tannins turn into crimson then into blue. In the presence of vanillin hydrochloric, condensed tannins give a red colouration.

E. Tannins binders development

Prepare tannin in water solution $N°1$ at 50% concentration and cassava flour in water solution N°2 at 30% on the tannin solids base then mix solution $N°1$ and solution $N°2$.The pH was adjusted to 10 with a potassium hydroxide (30%). Finally add 5% of hexamine solution (30%) on the tannin solids based.

Two types of binders based on the tannin of Ficus Sicomorus and Azadirachta Indica were manufactured; their various components are distributed in the table 2.

Table 1 : Species used

Class	Family	Usual name	Scientific name
Dicots	Moraceae	Fig	Ficus sicomorus
	Rhamnaceae	Mandermae	Zizyphus mandermae
	Meliaceae	Neemier	Azadirachta indica

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Table 2: Components of binder

Solution 1		Solution 2		Solution 3	
Principal element (50%)	**Solvent (50%)**	**Charge (30%)**	**Solvent (70%)**	**additives**	
Tannins	Water	Cassava flour	Water	KOH (30% on the tannin solids base)	Hexamine (30% on the tannin solids base)

III. RESULTS AND DISCUSSION

A. Results

Dose of tannin

The dosage of tannin permits to classify these three species in two groups:

- The first is composed with Ficus Sicomorus which content in percentage of dried barks is 36%;
- The second is composed with the Azadiratcha Indica and Zizyphus Mandermae less rich in tannins than the first group, their content in tannin varies between 11 and 12%.

They contain tannin are in the table 3.

These contains are representing by the following histogram :

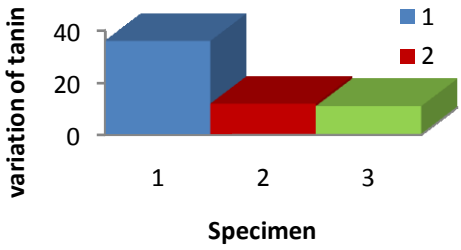

Fig 1 : Histogram representing the tannin variation in function of each species

On the x-axis: $1 = E1$ $2 = E2$ $3 = E3$

The areas occupied by the different species are set in accordance with the following below:

Table 3: Surfaces occupied by different species

E1	E2	E3
7%	15%	28%

The areas occupied by these species are materialized by the following sectors:

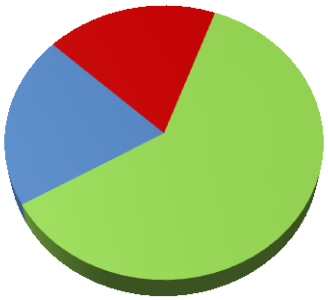

Fig 2: Sectors representing the surfaces used by the different species

Characterization

The adding of $1ml$ *ferric chloride* in a quantity containing crush barks (5 grams) gives a red and black colouration, characteristic of the presence of tannin.

To identify the *flavonoids,* just add to the extract $2\,ml$ of an alcoholic solution (*ethanol* +

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chloride of magnesium$50:50$*)* that will be followed by effervescence, the iron chloride gives a yellow *colouration*. The addition of iron *chloride* in a watery solution containing dried barks gives a *blue and black* precipitates showing the presence of ellagic and gallic tannins **[3];** *Green and black* in presence of *catechic tannins* and *blackish* in the presence of the mixture of ellagic , gallic tannins and catechic tannins **.**

The table 5 summaries the components present in each species of tannins.

The Phytochemical screening shows that the three samples $E1, E2, E3$ contain flavonoids, carbohydrates, amino acid, imino acid, pyrogallol, while only the sample $E1$ has the leucoanthocyanins, $E1\ and\ E2$ contain sweet substances, $E1$ does not contain gallic acid and digallic; $E2$ contains pelagic acids ...

Extraction

To extract each tannin, soak $500\ g$ of Ficus Sicomorus barks, $500g$ of Zizyphus Mandermae and $1\ kg$ of bark Azadirachta Indica in the solvent.

The first step of extraction with normal water described above leads to black and reddish liquid and solid remains; after evaporation, the washing with ethyl acetate + acetone permits to eliminate sweet substances.

Tannins binders development

Between those three tannins, two binders were manufactured: a binder based on tannins from the barks of Ficus Sicomorus and Azadirachta Indica. We find out that only the binder of the tannin of Ficus Sicomorus has good features: glues perfectly wooden materials, plastics, metals. Its shortest gel time ranges from 152 and 50 seconds for $pH = 10$, on the other hand the shortest gel time for the same experimental in the case of Azadirachta Indica range from 280 and 120 seconds and requires a relatively high pressing force to link objects.

The figure 3 shows the gel time of the binder based on tannin of Ficus Sicomorus in function pH, when Ph ranges from 7 to 14.

The main objective was to obtain a gel time less than 200 seconds , it is clear from our analysis that the shortest gel time [**4**] range from 152 and 50 seconds for $pH = 10$.The gel time changes with Ph; the higher the pH , the higher the gel time is short. For $PH > 11$, gel time increases again.

B. Discussion

The condensed tannins are used in many industrial applications: adhesives for wood products or dyes for leather **[5]**. They are mainly composed with repeat units of flavan- 3 -ol, flavan- $3,4$ -diol as profisetidine, prorobinetidine, procyanidin, prodelphinidin, and few fractions of simple sugars and polysaccharides, carbohydrates, so as traces of amino and imino acids [**5].**

According to Roux et al.[**1975],** only flavonoids of flavan - $3-$ ol and flavan- $3,4$ -diol help in the formation of condensed tannins. The condensed tannins are very interesting in the adhesive preparation because their polymeric networks formed are dense and present good characteristics.

The three samples $E1, E2, E3$ do not have the same components. The sample $E1$ contains flavan 3 ol, flavan $3,4$ diols; carbohydrates. The presence of simple carbohydrates (hexoses, pentoses and disaccharides) in great quantity of tannins influences the physical and chemical features of the binder because they increase viscosity and reactivity[**5].**

The flavonoids are also useful because the carboxyl group in position 4 of other types of flavonoid reduces nucleophilic characteristic and occupies one of the condensation positions **[5].**

In accordance with pictures 1, the tannin content of the $E1$ sample (36%) is one of the favourable assets for using the tannin as adhesives.

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Only its geographical distribution militates in its disfavour.

Among the most useful substances for the manufacturing of adhesives such as flavan 3 ol; the flavan $3,4$ diol; the carbohydrates; the $E2$ Sample contains only flavan 3 ol and some traces of carbohydrates and E3 contains only the flavan 3, 4 diol and some traces of carbohydrates.

The absence of flavan 3 ol (for $E2$) and the flavan 3, 4 diol (for E3) reduces the characteristics of good adhesive. The weak percentage in tannin ($11-12\%$) of $E2\ and\ E3$ samples also limitates their usage as raw material for the development of an adhesive on an industrial scale.

The $E2$ Sample contains hydrolysable tannin formed with elements such as gallic and digallic acids, the pyrogallol and pelagic acids, their presence have been identified through blue and black precipitates in the presence of iron chloride**[2].**

It is because of the weak supply on the international market and the weak reactivity with the formaldehyde that the usage of hydrolysable tannins (Gallic, digallic) is limited in the development of adhesives.

The falling gel time for $pH < 11$ could be justified by the fact that polymerization prevails in basic medium.

The increase gel time for $PH \geq 11$ observed in the binder developed with the tannin of Ficus Sicomorus could be justified by the fact that depolymerization prevails in very basic conditions [6].

The shortest gel times ranges from $152\ to\ 50$ seconds are slightly below the results obtained by other researchers in the formulation of adhesives mimosa tannin: $PH = 10$ with a percentage of silica $2\ to\ 6\ \%$ where the shortest gel times ranges from 90 to 190 seconds [1]:this means that the presence of tannins Ficus Sicomorus, potassium hydroxide polymerizes quickly relative to silica.

The extraction method using water has been adopted because it permits to obtain a better, raw and clear extract. From the raw extract, the washing with acetone + ethyl acetate permits to eliminate sweet substance and obtain pure tannin

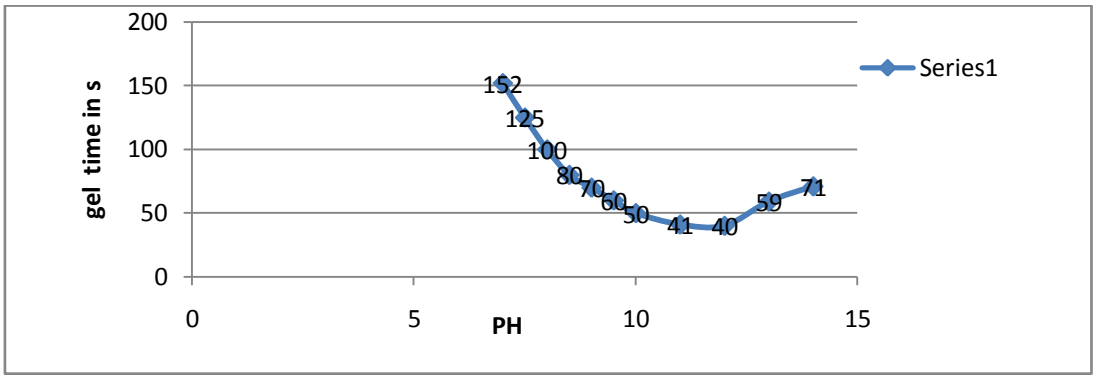

Fig 3: Gel time in function Ph ranges from 7 to 14

Table 4: Tannins content

Wood	Tannin content in % of dry bark
Ficus Sicomorus	36
Azadiracha Indica	12
Zizyphus Mandermae	11

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Table 5: Summary of tannins components

Components	E 1	E 2	E 3
Flavonoids	+	+	+
Flavan 3 ol	+	+	-
Catechol tannin	+	+	-
Gallic tannin	+	+	+
Flavan3, 4 diols	+	-	+
Flavon	+	-	+
Flavonol	+	+	-
Flavonon	+	+	+
Catechin	+	+	+
Leucoanthocyanins	+	-	-
Amino and imino acid	+	+	+
Gallic and digallic acids	+	+	+
Pelagic acids	-	+	-

Note: E1 = ficus sicomorus; E2 = zizyphus mandermae; E3 = azadiratcha indicha

+: Positive test; - : Negative test

IV. CONCLUSION AND PERSPECTIVES

It emerges from the characteristics commented above that among the three samples namely: Ficus Sicomorus, the Azadirachta Indica and Zizyphus Mandermae, only the tannin of the Ficus Sicomorus barks possesses all the necessary features to develop the adhesives: presence of flavan 3 ol, flavan 3, 4 diols, carbohydrates, medium tannin content (36%)….

These characteristics confirm the usage of Ficus Sicomorus sap as gum for our villagers. It now remains to develop an adhesive with a great quantity of these tannins to be used in gluing woods, papers and to think about multiplying the species of plants (under-represented, 15%) in the the sahelo - Sudanian area so that we will not suffer from any doubt as far as of its tannins in adhesives industries.

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After his PhD in Mechanical and Civil Engineering at the National Advanced Higher School of Cachan - France, DANWE RAIDANDI was from 1990 to 1993, monitor and research associate at the University of Paris 13; in June 1993, he was recruited as an assistant at the National Advanced School of Engineering of Yaounde - Cameroon; then promoted as lecturer and master lecturing. Today, he has **20**$^{+}$ of teaching experience. From 1995 to 1999, he was promoted Director of the research laboratory for automation at National Advanced School of Engineering of Yaounde - Cameroon; from 1999 to 2002, he was the Head of Department of Mechanical Engineering and automation at the IUT Douala. From 2013 to 2014, he was appointed Head of Department Coordination and Promotion of Research at the National Advanced School of Engineering of Yaounde; Since February 2014, he is the Director of the Graduate Institute of the University of Maroua. 42 reports of Engineers, 15 reports of masters research, 03 PhD reports were supervised by DANWE RAIDANDI and 08 PhD reports are ongoing. He participated at 15 national seminars and 39 international seminars and conferences.

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