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Composition and Binding Properties of Mucilages from Stem Bark of *Grewia venusta* and Calyx of *Bombax costatum*, two Tropical Plants Growing Wild in Togo

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Abstract

The stem bark of *Grewia venusta* and the flower sepal of *Bombax costatum*, two mucilaginous plants, were investigated as potential sources of bioadhesives for formaldehyde-free particleboards. Mucilage and pectin fractions of both plant organs were analysed for monosaccharides identification and quantification. The binding properties of these mucilages were investigated by testing mechanically particleboard made with the extracted mucilages. The aqueous extraction and ethanolic precipitation, followed by ionic chromatography gave the following results. Depending on the plant organ, extraction yield varied from 12% to 45 %. The pectin from *B. costatum* contained mainly rhamnose and arabinose (85%), while *G. venusta* bark's pectin enclosed basically arabinose, glucose and fucose (80%). The mechanical behaviour of the panels met most of the requirements of ANSI 208.1 standard.

Keywords: *Bombax costatum*, *Grewia venusta*, Pectin, Mucilage and Monosaccharides

Introduction

Mucilage is part of the plant polysaccharides fraction capable of becoming viscous by swelling in water, and *in vivo* it serves as a water reservoir. Its structural role in the plant includes ensuring thickening, adhesive and softening properties. Mucilage is widely used by rual populations in several countries, namely as natural active ingredients to purify and clarify drinking water. It is also used as adhesive to enhance the adhesiveness of lime in murals and in lime mortar (Cardenas, *et al*. 1998). Pectin is another mucilaginous substance not soluble in water, where it disperses in very fine particles. Pectin is present in many plant parts like tuber, fruit, stem bark, etc. and is often used as thickener in jams and emulsifier in mayonnaises and in sorbets, As dietetic fibres, mucilage and pectin are involved in the control of the cholesterol level in blood plasma (Lucas, *et al.* 2004) and glycaemia (Aller, *et al*. 2004; Ziai, *et al.* 2005).

The proven toxicity of formaldehyde to humans (Collins and Lineker, 2004; Kim and Kim, 2004) has prompted research throughout the world to provide manufacturers with safer and environmental friendly alternative adhesives for the manufacture of indoor building materials like particleboards, where the conventional adhesives are the formaldehyde-based phenoplastes (phenol-formaldehyde) or aminoplastes (urea-formaldehyde). Polysaccharides such as starch are known for their applications in various materials domains. Adhesive properties of starch modified by physical, chemical or physicochemical way were known for their use as matrix in composite applications (Garcia *et al.* 2001, Del Valle *et al.* 2005, Shibata *et al*. 2006). In our attempt to contribute to the development and promotion of natural adhesives without any or with low formaldehyde emission, based on plant binders, we have been investigating relevant organs of some typically tropical plant species known to contain biomolecules with binding properties including mucilaginous plants like *G. vensuta* and *B. costatum.*

The aim of this study was to undertake a prior chemical characterization of the potential mucilaginous bioadhesives through identification and quantification of the monosaccharide constituents in *B. costatum* sepal and in *G. venusta* stem bark. Both plant organs may provide substances with binding properties that could be probably used as natural and biodegradable adhesives for the manufacture of ecological particleboards with zero emission of formaldehyde.

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Materials and Methods

Plant material

The plant material used in this study consisted of particles from the core of *Hibiscus cannabinus* (kenaf) and dried powder of *B. costatum* flower sepals aned *G. venusta* stem bark as binding matter. These plant specimens were identified by Professour Akpagana team and voucher were deposited at the herbarium of the "Departement de Botanique" of the " Facultedes Sciences" at the Universite de Lome" in Togo. The *H. cannabinus* was obtained from an experimental cultivation, carried out from May to August 2005 at the "Station d' Exprerimentation Agropedagogique" (SEA) of the "Ecole Superieure d' Agronomie, Universite de Lome" in Togo. The stem bark of *G. venusta* was harvested on March 17th 2005 from wild growing plants in a locality of the Togolese savannah ecological area located at 6.42-8.4 degree northern latitude and 0.5-1.60 degree eastern longitude. The flower sepals of *B.costatum* were bought in a local market in Lome.

Determination of crude protein, cell wall constituents and total fat

The determination of the crude protein content was performed according to the conventional Kjeldahl method.

The dosage of the cell wall constituents (cellulose, hemicelluloses and lignin) was performed according to the conventional gravimetric method of Van Soest and Wine also known as ADF-NDF (Acid Detergent Fiber-Neutral Detergent Fiber) method.

The total fat content was determined through extraction with an extractor of the ASE DIONEX type using a mixed cyclohexane-water 95/5 v/v solvent at 100^{O}c (4 cycles of extraction; pressure of 120 bars), evaporation of the organic extract to dryness (rotatory evaporator) and ultimate oven drying (2 hours at 105^{O}c). Extraction trials were all performed in triplicate.

Extraction of mucilage

A sample of 150g of dried fine powder of plant material was mixed with demineralised water (4500 ml for *G. venusta* stem bark powder and 600 ml for the sepal powder of *B. costatum*). The suspension thus obtained was boiled for 1 hour, cooled at room temperature (2^{O}C), centrifuged for 15 minutes at 5000 rpm, the gelled solution collected and finally freeze-dried.

Extraction of pectin

A sample of 50g of plant material (fine dried sepal powder of *B. costatum* or dried stem bark powder of *G. venusta*) were macerated in 500 ml of demineralised water for 24 hours at 20^{O}C (Cardenas, *et al.* 1997; Saenz, *et al*. 2004). The resulting aqueous macerate was centrifuged (25^{O}C; 5000 rpm: 15 min), the supernatant filtered and mixed with 80% aqueous ethanol untill precipitation of pectin. The mixture obtained was again centrifuged (3000 rpm; 25^{O}C; 20 min) to yield a precipitate, which was finally freeze-dried as above or oven-dried (70^{O}C; 48 hours). Experiments were carried out in triplicate.

Hydrolysis of mucilage and pectin

The mucilage and pectin obtained as above mentioned were hydrolysed for monosaccharides identification and quantification. Thus a 5g sample of mucilage or pectin was reacted in 500 ml erlen flask with 150 ml H_2SO_4 (72% m/m; 12 M) for 30 minutes at room temperature (approximately 25^{O}C). In the second phase of the hydrolysis process, and for each reaction duration tested, a sample of 5 ml of this acidic reaction mixture were taken in 50 ml test tube, then diluted to 2 M with 25 ml of demineralized water, heated to 100^{O}C and kept at this temperature in water bath for varying durations (15, 30, 45, 60, 90 and 120 minutes) in order to study the kinetics of the monosaccharides formation. This hydrolysed solution was cooled, neutralized with NaOH 32% (m/v_), centrifuged, (5000 rounds.min^{-1}, 15 min) (Davis, 1998) and the supernatant recovered for dosage by High Pressure Liquid Ionic Chromatography (HPLIC). Experiments were carried out in triplicate.

Determination of the glucuronic acid

The content in glucuronic acid was obtained according to the method developed by Blumenkrantz and Asboe-Hansen, 1973, which includes an acid hydrolysis of the mucilage or pectin and their subsequent dosage by UV spectroscopy at 520 nm.

Determination of sugar components

The separation and quantification of sugar components was performed on aliquots of the supernatants yielded by the double hydrolysis of mucilage and pectin using HPLIC. The analysis was carried out on a chromatographic chain of the DIONEX BIO DX 300 type equipped with a CarboParc PA1 type column. The later is recommended for monosaccharides compositional analysis, linear homopolymer separtion and saccharides purification. Its resin consisted of a 10- µm diameter polystyrene/diviny1benzene substrate agglomerated with 350- nm MicroBead quaternary amine functional-

ized latex (5% cross-linked) and had the following other characteristics: (i) anion exchange capacity: 100 µeq per 4 x 250-mm column; (ii) flow rate: 1 ml/min (4 x 250-mm column) (iii) pH compatibility: 0-14; (iv) organic solvent compatibility: 0-2 %, (vi) Maximum back pressure: 28 MPa. The elution was achieved by a system of three eluents which were: NaOH 1 M (E1), Water UHQ (E2) and NaOH 100 mM (E3) with the following gradient of elution: 99% (E2) and 1% (E3) for the first 30 min, 100% (E3) from 30 to 39 min and 100% (E1) from 39 to 50 min. The standards used were commercial arabinose, galactose, rhamnose, fructose, maltose, sucrose, fucose, glucose, and mannose from FLUKA with more than 99% purity.

Preparation of kenaf core particles

Laboratory scale particleboard manufacturing

The chopped kenaf core was oven-dried (70 ^{O}C, 48 hours) and milled into particles using a RETSCH SM 100 type crusher equipped with a 5 mm mesh sieve, which gave 67% of particles with diameter varying from 0.25 to 1.60 mm, length from 1 to 7 mm and variable fibres length from 5 to 19 mm.

The binders used in particleboard manufacturing were freeze dried mucilages extracted from *B. costatum* calyx and stem bark of *G. venusta* on the one hand and Urea Formaldehyde resin, (106, liquid: viscosity at 25^{O}C: 90 cp; dry matter content: 65%; Sp Gravity: 1.286 and pH:8), purchased from Chimar Hellas S.A. (Greece) on the other hand.

For each panel manufacturing, the binder (powder freeze dried mucilage of *B. costatum's* calyx or *G. venusta's* stem bark or liquid UF resin) was incorporated to the kenaf core particles in the rate of 10% on the basis of fibre dry matter. The mixture of kenaf core particles and binder (300 g dried matter) was humidified with 40 g of the demineralised water. The mixture was firstly mixed manually during 2 min then mechanically during 10 min using a PERRIER 721 mixer.

The mat thus obtainced was thermal pressed in an aluminium mould (27 cm x 27cm x 5 cm) placed between two electrically heated (108^{O}C) plates of a manual hydraulic thermal pressing machine of the CARVER type (maximum pressure: 11 tons.m^{-2}) and the pressure was gradually applied as follows: 5 tons^{-2} for 60 seconds and then 10 tons.m^{-2} for 4 minutes. The panel was then removed from the mould and weighted after cooling.

Mechanical testing of the particleboards

6 specimens measuring 150 mm x 50 mm and 6 specimens (50 mm x 50 cm) were cut from each particleboard according to the NF-EN 326-1 (1993) standard and conditioned at 20^{O}C, 65% relative humidity for 14 days before testing. The 150 x 50 specimens were used for bending testing according to the NF-EN 310-1 (1993) standard and the 50 x 50 specimens for internal bond strength testing on in accordance with the NF-EN 319, (1993) standard requirement. For both of the mechanical tests, a JFC type H5KT testing machine was used.

Data statistical analysis

STATISTICA V5.5 software was used for the statistical analysis of the data

Results and Discussion

Gravimetric characteristics of the tested plant organs

The plant organs studied in this work were basically made of carbohydrate polymers including cellulose and hemicelluloses (a total of 62.40% in the sepal of *B. costatum* and 71.44% in the stem bark of *G. venusta* of the dry matter) (Table I). The crude protein content of our *B. costatum* sample was very close to that reported (7.8%) by Glew *et al*. 1997 for the same plant organ. The total mineral was higher than that reported by the same authors (2.0%); but they found higher content of fatty matter (2.5 %). The broad variability of the chemical composition of plant material is a well known phenomenon related to various factors. We could not find any literature reports for comparison to our findings on *G. venusta* which is most probably a lesser known mucilaginous species.

Table I: Main components of tested plant organs

Content (%)	Tested plant species (organ)	
	B. costatum (sepal)	*G. venusta (stem bark)*
Mineral matter	5.47	9.55
Crude protein	7.87	4.06
Fatty matter	0.83	1.14
Crude cellulose	22.58	50.32
Hemicelluloses	39.82	21.12
Lignin	4.95	3.82

Chemical components were quantified as a percentage of the total dry weight.

Yield in mucilage and pectin

The content of our plant organ samples in water extractible was species-dependent. Indeed, the yield of mucilage extraction was 20% and 45% respectively for *G. venusta* and *B. costatum* (Table II). In the same manner, *B. costatum* contained much pectin (33.7%) than *G. venusta* (12.0 %), These extraction yields were very high as compared to previous findings on other mucilaginous plant species yield in mucilage: 9-19 % for *Opuntia ficus indica* cladodes (Saenz, *et al.* 2004); 24.9% and 17.7% in *Prosopis chilensis* seed depending on the extraction method (Estevez, *et al.* 2004).

3.3 Carbohydrate content in tested plant organs

The yields of glucuronic acid from the hydrolysis of mucilage and pectin reported in Table II show that the contents depended on the plant species. Indeed, the hydrolysis of freeze-dried mucilage of *B. costatum* yielded more glucuronic acids (58.03%) than that of *G. venusta* (49.73%). Conversely, the hydrolysis of freeze-dried pectin of *G. venusta* yielded more glucuronic acid (61.12%) than that of *B. costatum* (49.15%). It could be assumed that the content in mucilage and pectin depened on the plant species as *B. costatum* seemed to contain more mucilage than *G. venusta.* Conversely, the later species appeared richer in pectin than the other one. Obviously too many parameters are capable of explaining this findings and at this stage of our investigation we could only link them to the difference of both plant species.

Our results compared with the yields (16.5 to 63% of dry weight, based on carboxylic group dosage) in galacturonic acid obtained by Vignon and Garcia-Jaldon, 1996 on hemp polysaccharides. But these yields in uronic acids were much higher than those claimed by several authors with different mucilaginous plant species: 8.7% on the fruit of *Cordia abyssinica* (Benhura and Chidewe, 2002), 4.4% in the mucilage of the fruit of *Zizyphus spina-christi* (Nazif, 2002), 6.3% in the spine of prickly pear (*Opuntia ficus-indica*) (Vignon, *et al.* 2004), 30% in the mucilage from the seed coat of *Chorisia speciosa* a Bombacaceae species (Beleski-Carneiro, *et al.* 2002).

The main identified sugars in the hydrolyzed pectin and mucilage are listed in Table III below. The pectin in the sepal of *B. costatum* consisted mainly of arabinose (57%) and rhamnose (28.71%). They were also found as main constituents in the mucilage of the same organ in addition to glucose (19.22%), mannose (14.86%), and fucose (8.57%). The most striking difference was the absence of glucose and mannose in the pectin. No previous experimental data could be found for discussion, but (Beleski-Carneiro, *et al*. 2002) found that the monosaccharides of the mucilage of the seed coat of *Chorisia speciosa* (Bombacaceae) were mainly galactose (54%), rhamnose (25%) and glucose (21%). In *B. costatum*, galactose was not detected in this study.

The pectin from the stem bark of *G. veusuta* yielded mainly arabinose (33.96%), glucose (31.47%) and fucose (15.25%), associated with little amount of rhamnose (5.97%), mannose (5.96%) and maltose (4.90%). The mucilage of this organ produced glucose (48.80%) and xylose (26.40%) as the major constituents.

Table II: Extraction yield and composition of mucilage and pection of *B. costatum* and *G. venusta.*

Sugar component	*B. costatum (%)*		*G. venusta (%)*	
	Mucilage	Pectin	Mucilage	Pectin
Extraction yield	45	33.7	20	12
Glucuronic acid	58.03	49.15	49.73	61.12
Rhamnose	12.21	28.71	8.74	5.97
Fucose	13.31	7.19	6.93	15.25
Arabinose	28.13	57.27	4.24	33.96
Glucose	19.22	-	48.80	31.47
Mannose	14.86	-	-	5.96
Xylose	8.57	1.18	26.40	-
Sucrose	3.70	-	3.14	-
Maltose	5.64	-	4.901	1.76

The overall observation was that the sugar composition of pectin differed from that of mucilage within the same organ. Moreover the sugar composition of pectin and mucilage seemed species-dependent.

Mechanical properties mucilages based particleboards

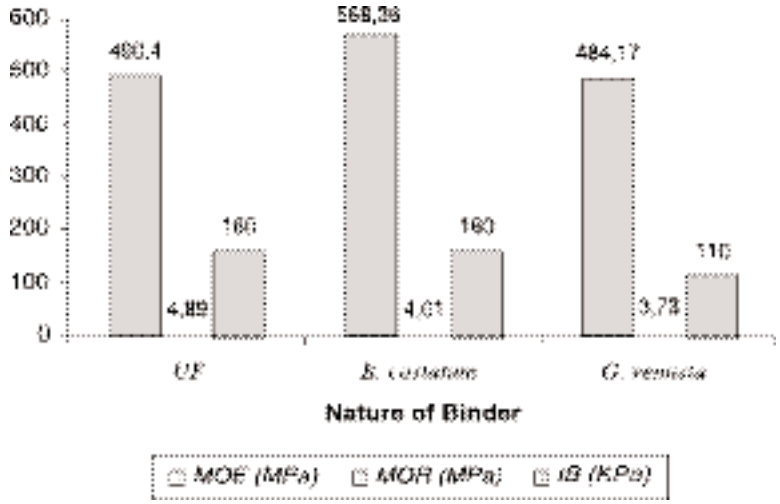
The particleboard obtained in this work was low density insulation type with an average density of 435.62+21.85 kgm^{-3}. This type of particleboard usable for ceiling and for wall coating is slightly higher than that obtained by Sellers et al. 1993 (256 $kg.m^{-3}$) on the kenaf core particleboards with Urea Formaldehyde (UF), Phemol Formaldehyde (PF) and Polymeric Diphenylmethane Diisocyanate (PMDI) resins.

Figure 1 shows variation of the particleboards mechanical's properties as function of nature of binder used for their manufacturing. Comparing the flexural modulus of elasticity (MOE), this figure revealed that specimen containing mucilage extracted from the calyx of *B. costatum* presented the highest modulus (565.36 MPa) while the specimens of panels with the two other types of binders: mucilage from stem bark of *G venusta* and a conventional particleboard resin, UF used as reference showed identical MOE (484.17 MPa and 490.40 MPa respectively). Of the three types of particleboards, only the panels containing mucilage of *B. costatum* reached the value required by the american standard ANSI 208.1 (550 MPa) for the MOE of this type of panels.

mucilage based panels (4.01 MPa and 3.73 MPa). The origin of the mucilage didn't have any influence on the MOR values of the panels.

All the particleboards made in these studies showed average Internal Bond strength (IB) properties equal or higher than that required by standard ANSI 208.1 (0.1 MPa) for the type of panels made. For this parameter, the mucilage from the calyx of *B. costatum* had induced on the panels an effect identical to that of UF resin. The IB value obtained with the panels containing the mucilage of *G. venusta* stem bark was slightly low compared those induced on the panels by the other types of adhesive.

We did not find experimental data relating to the specific use of mucilages as binder in manufacture of particleboards. Nevertheless, Shibata *et al*. 2006 working on hot press forming composites manufactured with corn-starch-based resin reinforced with 60% of fibers from two types of kenaf, obtained on their panel, flexural properties very high compared to ours (MOE : 4 to 4.5 GPa; MOR : 40 to 65 MPa). This significant superiority of the results obtained by these authors, compared to ours can be explained by (i) the proportion of resin in the panels which was 40% for Shibata *et al*.



UF: Urea formaldehyde resin: MOE: Bending modulus of elasticity; MOR: Bending strength; IB: Internal bond strength

Figure 1: Effect of binding mucilaginous extracts on mechanical behaviour of kenaf based particleboafds

The bending strength properties (MOR) of all the specimens of the panels obtained met the ANSI 208.1 standard requirements (3 MPa). Comparing the effect of the types of adhesives on the bending strength, the conventional binder (UF) showed the best average value of the MOR (4.89 MPa) which was statistically higher than those observed with

specimen against 10% in our panel; (ii) the matrix that we used was not submited to any improving modification; and (iii) we used kenaf core distinctly less dense and thus highly more compressible than the fibres employed by the author (Shibata *et al*. 2005 revealed that fibre compression affected considerably the flexural modulus.

Conclusion

In view of their possible used as bioadhesives in ecological particleboards, the water extractible carbohydrates isolated as pectin and mucilage from the sepal of *B. costatum* and the stem bark of *G venusta* both tropical mucilaginous plant species growing wild in Togo, have been analysed for their pectin and mucilage monosaccharides identification and percentage composition. Thermogravimetric determination showed that cell wall constituents totalled 67 per cent and 75 per cent respectively in the sepal of *B. costatum* and the stem bark of *G. venusta*. Depending on the plant species and the extraction method, the content of tested plant organs in water extractible carbohydrates varied from 12 percent to 45 percent. Recovery of extracted pectin and mucilage was best achieved by freeze-drying than oven-drying. The major monosaccharides identified in the sepal of *B. costatum* were rhamnose, fucose, arabinose, glucose, mannose and xylose. The stem bark of *G. venusta* contained mainly fucose, arabinose, glucose, and xylose. The mucilages extracted were used as matrix for manufacturing of low density kenaf core particleboard (435.62+21.85 $kg.m^{-3}$). Mucilages based panels revealed mechanical characteristics relatively weak but equivalent to those induced by the UF. Independently of the nature of the matrix used, the bending strength and the internal bond strength of all of the elaborated specimens reached the ANSI, 208.1 standard requirement. But on the other hand, only the panels containing mucilage from calyx of *B costatum* got a MOE in conformity with this standard.

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