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- Polymer chemistry.
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- Polymer physics.
- Polymer thin films and membranes
- Polymeric hybrid materials.
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Characterization of Exudates from *Araucaria Heterophylla* and *Afraegle Paniculata* Exudates, Two Ligneous Species from Beninese Flora for Bio-Sourced Polymeric Materials Search

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Abstract Plant exudates have been of therapeutic interest since immemorial time, but very few are scientifically known to be exploited, as is the case with exudates from *Araucaria heterophylla* (Ah) and *Afraegle paniculata* (Ap), two multi-purpose species found in Benin's flora. This first study is a scientific survey of the various properties of these two exudates with a view to exploiting their potential. After harvesting the two exudates, were submitted to physicochemical, chemical, and physical analyses. A solution test was used to identify the correct solvents and to assess the dispersibility of insoluble or low-solubility exudates. Phytochemical screening was carried out to determine the major metabolic groups present. Thermal analyses (TGA and DSC) were used to assess degradability and thermal transitions; Fourier Transform InfraRed spectroscopy was used to identify chemical functions. Results show that exudate Ap is water-soluble and both exudates are soluble in dimethylsulphoxide and disperse perfectly in polar aprotic solvents such as acetone or protic solvents such as ethanol. Exudate Ah is solid and brittle while Ap is liquid, unctuously sticky, and viscous. Infrared spectroscopy confirmed the presence of alcohol and carbonyl functions and glycosidic bonds. Phytochemical screening showed the presence of reducing sugars, mucilages, saponosides, terpenes, and sterols. Both exudates underwent thermal degradation at around 300°C, preceded by dehydration at 100°C. Ap exudate shows thermal transitions such as melting (Tm = -10°C; ΔHm = 65.30 J. g^{-1}), crystallisation (Tc = -54.2°C; ΔHc =11.35 J.g^{-1}). As for the Ah exudate, it exhibits a glass transition at 17±1°C. The conclusion is that exudate Ah is a totally amorphous solid and Ap is a crystalline solid or liquid as function of the temperature.

Keywords: *Exudate, bio-sourced, polymers, Afraegle paniculata, Araucaria heterophylla*

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1. Introduction

Plant exudates are mixtures of complex organic compounds that exude from the pores of a tissue as a result of external stress caused by insects, humans or wind. They are made up of mixtures of complex molecules, mainly polysaccharides [1]. Polysaccharides are biological polymers made up of one or more monosaccharide units linked together by osidic bonds. There are two categories, depending on their biological function: structural polysaccharides (cellulose, pectin), which are involved in the formation of organic structures, and reserve polysaccharides (starch, glycogen), which are the main sources of energy for living organisms [2]. Traditionally, plant exudates have been used to treat various diseases due to their antiseptic and anti-inflammatory properties [3,4].

Their use in the food, pharmaceutical, cosmetic, and other industries, for their physicochemical and biological properties, has made them internationally prized products whose demand is constantly increasing [5]. Benin's flora is

a reservoir of biodiversity that plays an important role in satisfying basic human needs. Due to the variability of its climate [6,7], various species thrive there, including food and medicinal species. There is an abundant variety of exudate-producing species, including *Afraegle paniculata* and *Araucaria heterophylla* from the Rutaceae and Araucariaceae families respectively [6]. The current uses of gums, as excipients in the administration of active ingredients and as gelling agents in food systems have made polysaccharides, biological polymers with broad advantages as opposed to synthetic compounds [8]. Because of their abundance, renewable nature, non-toxicity, and biodegradability, these biopolymers have very interesting properties for the formulation of biodegradable polymeric materials and food and pharmaceutical auxiliaries. In the current context of research into new sources of active ingredients and new value-added products, this first section of the study of the plant gums of *Afraegle paniculata* and *Araucaria heterophylla* from Benin consists of assessing the physicochemical, chemical, and thermal potential of the exudates of these two species, which are little known in the scientific world but have considerable therapeutic and food value.

2. Materials and Methods

The plant material consists of the exudates of two plant species: *Afraegle paniculate* (Schum.) (Ap) and *Araucaria heterophylla* (Salisb.) (Ah) (Figures 1 and 2).

Figure 1. *Afraegle paniculata* (a-Tree; b-Fruit; c-Exudate)

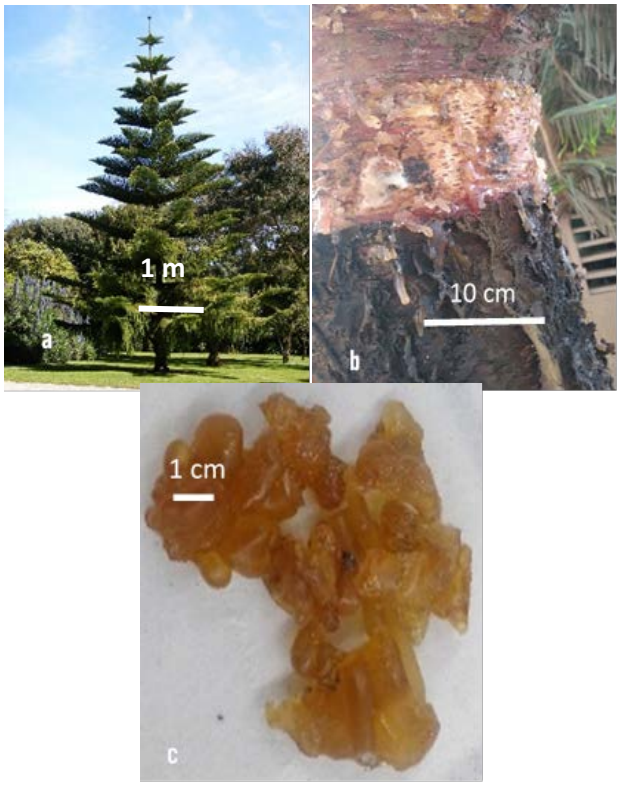

Figure 2. *Araucaria heterophylla* (a-Tree; b-Exuded bark; c-Exudates).

Ap exudate was extracted from the mature fruit of *Afraegle paniculata* which was collected in Natitingou in northern Benin. Ah exudate was collected by incision from the bark of the branches and trunk of the *Araucaria heterophylla* tree at Togoudo in the Abomey-Calavi commune in southern Benin. Both exudates were harvested in the dry season (October to November).

2.1. Organoleptic Test

The exudates were submitted to various organoleptic evaluations including color, odor, shape, taste, and texture. The majority of information based on the identification of exudate purity and quality can be derived from these observations [8].

2.2. Phytochemical Analysis

Secondary metabolites were identified by specific reactions of staining and precipitation for each family of metabolites [9,10,11].

2.3. Determination of Non-Volatile and Volatile Extractives in Exudates

Polysaccharides were extracted from exudates by the method of Samrot [12] with some modifications. 50 g of exudates were dispersed in 200 ml of ethanol for 12 hours to remove the oily phase. The powder collected, after filtration on Wattman paper, is dissolved in 100 ml of demineralized water under magnetic stirring and at room temperature. The viscous solution obtained was heated

to 100°C for 1 hour in the presence of 10% (w/w) trichloroacetic acid by volume of the solution, then centrifuged after cooling at 10500 rpm for 15 minutes. The supernatant collected was treated with acetone to precipitate the polysaccharides. After centrifugation, the solid phase obtained was dried under vacuum at 45°C until constant weight. Polysaccharide content (Pc) was assessed by calculating the extraction yield in relation to the crude exudate using equation (1):

$$Pc = \frac{m_s}{m_0} * 100 \quad (1)$$

Where m_s = mass of dry polysaccharides; m_0 = mass of crude exudate.

2.3.1. Protein Content

The proteins recovered by centrifugation during polysaccharide extraction are dosed by the Bradford method. A calibration curve is prepared first at 595 nm using a range of aqueous BSA (Bovine Serum Albumin) solutions with concentrations within the range of 2 to 10 µg. mL^{-1}. To prepare the sample, 1.5 g of the protein fraction was dispersed in 4.5 ml of 10 mmol. L^{-1} Tris-HCl buffer solution at pH 8. The assay was carried out according to the method described by Harisson et al., [13]; Shepard & Bradley [14].

2.3.2. Ash Content.

The ash content (Ac) was determined by incineration at 550°C in a muffle furnace until the ash residue was weighed constant [15,16]. This content was estimated by the equation:

$$Ac = \frac{m_2 - m_1}{m_{ech}} * 100 \quad (2)$$

Where m_2 = final mass (crucible + ash) obtained after incineration; m_1 = mass of empty crucible; m_{ech} = mass of exudate before incineration.

2.3.3. Volatile Compound Content.

The essential oils were extracted by hydrodistillation using a Clevenger-type apparatus, from a mass of between 150 and 200 g of exudate in a round-bottomed flask containing a sufficient quantity of water to cover the exudate. The extraction yield of the essential oil (EO) as a function of the plant material is then expressed by the following formula:

$$EO = \frac{m_f}{m_i} * 100 \quad (3)$$

With m_f = mass of collected oil; m_i = mass of introduced exudate and EO extraction yield

2.4. Solution Test for Plant Exudates

The exudates were submitted for solubility study at room temperature (19±1) °C in different solvents such as demineralized water, ethanol (protic polar solvents), acetone, dimethyl sulphoxide (aprotic polar solvent), and hazelnut oil (apolar solvent). 0.5 g of plant exudate was dissolved in 10 mL of each solvent under magnetic stirring for 24 hours, followed by measured additions up to 100 mL for at least 72 hours.

2.5. Thermal Analysis

Approximately 10 mg of exudate, in an aluminium crucible crimped under a hydraulic press, was submitted to a temperature variation of 10°C.min^{-1} from 25 to 600°C. Analyses were carried out under nitrogen at a flow rate of 50 ml.min^{-1}; mass losses were analyzed using a Mettler Toledo TGA/DSC1 thermogravimetric analyser.

The various thermal transitions were determined on approximately 10 mg of exudate using a TA Instruments DSC Q 10 calorimeter, with a temperature rise from -80 to 150°C followed by cooling from 150°C to -80°C at 10°C.min^{-1}, separated by a 3-minute isotherm.

2.6. Fourier Transform Infrared Spectroscopic Analysis

The chemical functions of the exudates were identified by reflection (ATR-Attenuated Total Reflectance) using a Thermo Scientific Nicolet iS50 FT-IR Fourier Transform Infrared Spectrometer (FT-IR). Liquid samples were deposited on the diamond tip of the ATR module and solid samples were transformed into KBr pellets using a hydraulic press. IR spectra (32 scans) were recorded at room temperature 19±1°C, over a wave number range of 500-4000 cm^{-1}. The spectral analyses were verified on 3 samples and the spectra were analyzed using OMNIC software.

2.7. Rheological Analysis

The rheological behavior of crude exudates and their polysaccharides in aqueous solution was studied using a hybrid rheometer, model HR20, with a Couette-type geometry controlled by TA Instruments TRIOS software, version V5.1.1. Shear viscosity of the samples was measured in steady shear flow conditions for various shear rate from 0.1 to 100 s^{-1}.

3. Results

3.1. Organoleptic Properties of the Exudates

Table 1 presents the organoleptic test results for the two exudates.

Table 1. Organoleptic Characteristics

Properties	Observation	
	A. heterophylla	*A. paniculata*
Appearance	Orange colour	Caramel colour
Odor	Pronounced	Hay odor
Flavor	Insipid	Sweet when ripe
Texture	Hard and brittle	Sticky, unctuous liquid

3.2. Phytochemical Analysis

As shown in Table 2, both exudates contain mucilage, reducing sugars, saponins, terpenes, and sterols. Tannins are revealed only in the *A. heterophylla* exudate.

Table 2. Secondary metabolites in Ah and Ap exudates

Secondary metabolites	A. heterophylla	A. paniculata
Mucilage	+	+
Reducing sugars	+	+
Tannins	+	-
Flavonoids	-	-
Anthocyanins	-	-
Leucoanthocyanins	-	-
Anthraquinones	-	-
Saponosides	+	+
Alkaloids	-	-
Coumarins	-	-
Terpenes and sterols	+	+

+ = revealed, - = not revealed

3.3. Extractives Content

Table 3 shows the composition of the two exudates in terms of polysaccharides, proteins and total mineral matter or ash.

Table 3. Extractives Content of Ah and Ap Exudates

Exudates	Polysaccharides (%)	Protein (ug/g)	Ash (%)	Essential oils (%)
Ah	50.7±2.0	7.6±0.2	3.0 ± 0.1	0.7±0.2
Ap	55.8±10.0	22.8±5.2	1.4 ± 0.1	0

Both exudates contain more than 50% polysaccharide biopolymers, the highest polysaccharide content 55.8% was obtained with Ap exudate, against 50.7% from Ah. In terms of protein, Ap contains three times more protein (22.8 µg of BSA equivalent per g of crude exudate) than Ah (7.6 µg of BSA equivalent per g of crude exudate). Physicochemical analyses of the exudates show 3.0 ± 0.1 % and 1.4 ± 0.1 % for the ash content respectively for Ah and Ap. Only the *Araucaria heterophylla* (Ah) exudate contained essential oil with a yield of 0.7 ± 0.2 %.

3.4. Solubility of Exudates

Table 4 shows the results of the exudate solution test at room temperature at 19 ± 1°C. Ap exudate is totally soluble in demineralized water at 100 $g.L^{-1}$.

Table 4. Exudate Solution Test

	Solvents	Type of solvant	Dielectric constant (ε) at 25°C	Ah	Ap
1	Distilled water	Polar protic	88	ps	S (100 $g.L^{-1}$)
2	DMSO	Apolar protic	45	ps 6,25 $g.L^{-1}$)	ps (6,25 $g.L^{-1}$)
3	Ethanol	Polar protic	24,3	D	D
4	Acetone	Polar aprotic	17,7	D	D
5	Oleic oil	Few Polar protic	2,5	D	D
6	Hazelnut oil	Apolar	ND	D	NS ; d

Legends: D = totally dispersed; S = totally soluble; NS = non-soluble, ps = weakly or partially soluble; DMSO = dimethylsulphoxide.

We observe that exudate Ap is completely soluble in distilled water at 100 $g.L^{-1}$ at an ambient temperature of 19±1°C. Both exudates were also soluble in dimethyl sulphoxide (DMSO) at 30±1°C at low concentrations. Both exudates were remained insoluble in acetone, ethanol, oleic acid and hazelnut oil but they were completely dispersed as fine particles in acetone and ethanol.

3.5. Thermal Properties of Exudates

3.5.1. Thermogravimetric Analysis

Figure 3 shows the thermogram of mass losses of the two exudates during heating from 25 to 600°C in a closed crucible. Thermogravimetric analysis showed that these exudates decomposed in two stages, the first of which, at 100°C, corresponds to the water evaporation estimated at 40% and 3% for Ap and Ah respectively. The second stage of decomposition, is centered around 300°C. the two exudates have a residue of at least 20% by mass of exudate introduced.

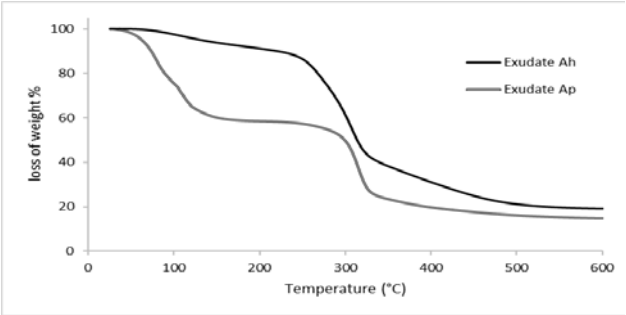

Figure 3. Mass loss thermograms (TGA) of Ah and Ap exudates.

3.5.2. Differential Scanning Calorimetry

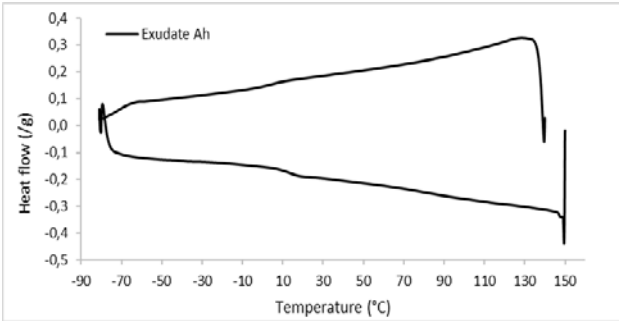

Figure 4. DSC thermograms of Ah exudates.

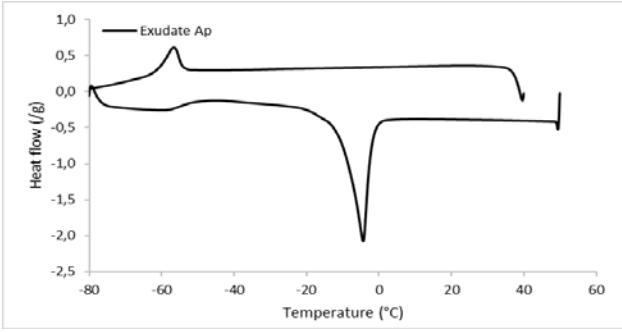

Figure 5. DSC thermograms of Ap exudates

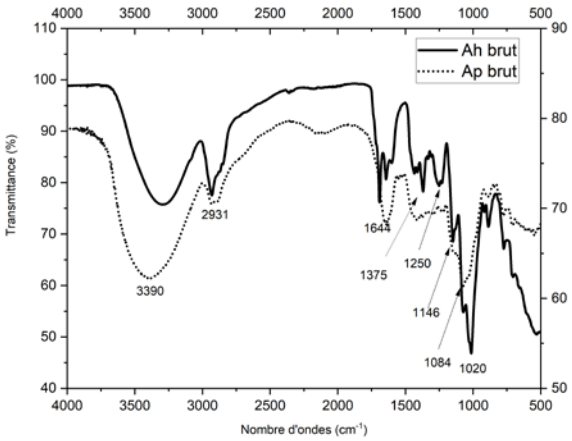

Figure 6. IR-TF spectra of *Afraegle paniculata* and *Araucaria heterophylla* exudates

Figures 4 and 5 show the DSC thermograms of the two exudates between -80 and 150°C (heating) and between 150 and -80°C (cooling) for exudate Ah, and between -80 and 50°C (heating) and between 50 and -80°C (cooling) for exudate Ap. For the Ap exudate, figure 4 shows a detectable inflection point at 18°C during the temperature rise and another at 16°C during cooling. For exudate Ap, in figure 5, there is an endothermic peak around -10°C during the temperature rise with an enthalpy variation of 65±5 $J.g^{-1}$. while during the cooling, an exothermic peak was obtained at -54.2°C with an enthalpy variation of 11.3 $J.g^{-1}$.

3.6. Fourier Transform Infrared Spectroscopic Analysis

Figure 6 shows the infrared spectra of the raw exudates.

The infrared spectrum of the two exudates shows common broad-band peaks around 3390 cm^{-1} and a weak peak around 2930 cm^{-1}. There is also a peak for Ap and two for Ah around 1644 cm^{-1} and a strong absorbance band around 1080 cm^{-1} for Ap and 1020 cm^{-1} for Ah with a shoulder. Additional peaks were obtained at 1375 and 1250 cm^{-1} for Ah.

3.7. Rheological Properties

Table 5 shows the apparent viscosity of exudates and their polysaccharides in aqueous solution, taking into account the water content of their exudates.

Table 5. Apparent viscosities of exudates and polysaccharides in aqueous solution of Ah and Ap

	Samples	Apparent viscosity (Pa.s) at $\dot{\gamma}$ =1s^{-1}, 25°C
Ap	Crude exudate	12.9
	Polysaccharids (40%w/w) in aqueous solution	0.69
Ah	Crude exudate	ND
	Polysaccharids (40%w/w) in aqueous solution	0.045

ND: not determined because Ah crude exudate not enough softened due to its Tg (17°C) lower than the temperature of analysis at 25°C.

The Ap exudate has a viscosity of 12.9 Pa.s. When solubilised at 40% in aqueous solution, in accordance with the water content in the crude exudate, the polysaccharides Ap and Ah extracted from this exudate have an apparent viscosity at 1 s-1 of 0.69 Pa.s and 0.045 Pa.s respectively. The results suggest that the molar mass of Ap exudate is greater than that of Ah one (at least 5 times greater).

4. Discussion

The organoleptic test carried out on the two exudates showed that Ah exudate is tasteless, hard and brittle, orange in colour and with a characteristic, pronounced odour; whereas Ap exudate, which is sweet when ripe, is liquid, unctuously sticky and has a hay-like odour. The sweet taste of Ap exudate when ripe, coupled with its attractive odour, explains why it is used in human food, unlike Ah.

Calorimetric analysis showed that melting and crystallisation thermal transitions for Ap are below the ambient temperature while for Ah exudate a glass transition is close to the ambient temperature.

The rheological analyses carried out on the exudates showed that, unlike Ah exudates and gum arabic, Ap exudate is capable of flowing.

Phytochemical analysis showed that both exudates contain various primary metabolites (lipids, proteins and carbohydrates) and secondary metabolites such as sterols and terpenes, mucilages and reducing compounds. In addition to these metabolites, tannins are also present in the Ah exudate. Previous studies have reported the presence of saponins, mucilages, and reducing compounds in this exudate [8] and terpenes and sterols [17-19]. The Ap exudate being studied for the first time in this work to our knowledge, has no comparative data in the literature. Compounds such as flavonoids, alkaloids, anthocyanins, coumarins, leuco-anthocyanins, and anthraquinones not found in Ah and Ap exudates in the present study have also been reported absent in the Indian species [8].

Both exudates exhibit partial (Ah) and total (Ap) solubility in aqueous media but are insoluble in organic solvents such as acetone, ethanol, and oleic acid. These results are consistent with those of Gayathri et al., [8] for exudate Ah.

The positive test for mucilages and reducing compounds would reveal a high presence of carbohydrates and polysaccharides with extraction yields relative to crude exudate of 51% and 56% for Ah and Ap, respectively. This 51% polysaccharide biopolymer content obtained in the Ah exudate harvested in Benin is still lower than the 67% yield found in the Indian species [12]. This difference in content may be linked to the harvesting period, the nature of the soil, or climatic factors.

The high-water content 40±0.2 % of the Ap exudate clearly explains its liquid nature in contrast to the Ah exudate, which has a content of 3 ± 1 %. This content is similar to value found by Gayathri [8] for Egyptian and Indian species as well the total mineral content found by these authors (i.e. 2%). Moreover, this content is lower than that reported by Thevernet [20] in his work on the two gum Arabic species, i.e., 2.80% for the Senegalese

species and 3.90% for the Seyal species. The differences in these results could be linked to species variety.

Of the two exudates studied, only Ah contained essential oil with an extraction yield of 0.71±0.22. This content is much lower than that reported by Elshamy [18] in their work on the species harvested in Egypt, i.e. 2.35% (v/w), which contains half as much essential oil as the Indian species [19], i.e. 5.70% (v/w). This variation in the essential oil content of the different species could be linked to edaphic conditions [8],[17],[19].

Fourier Transform infrared spectroscopy revealed a broad peak around 3390 cm^{-1} and a weak peak around 2930 cm^{-1} attributable to stretching and bending vibrations of the O-H and C-H groups [21][22]. a peak at 1644 cm^{-1} and a strong absorbance at 1080 cm^{-1} for Ap and 1020 cm^{-1} with a "shoulder" for Ah corresponding to vibrations attributable to the C=O [21][23] carbonyl and C-O etheric and C-C groups [24].

5. Conclusion

Phytochemical screening of *Afraegle paniculata* exudate, characterised for the first time in the present study, shows that this exudate, like that of *Araucaria heterophylla*, is rich in secondary metabolites such as mucilages, reducing sugars, saponins, terpenes, and sterols. The sweet flavor of the mature Ap exudate, its high water content, and its attractive odor would justify its liquid and viscous nature and its use in human food, unlike Ah. This study also showed that both exudates are rich in polysaccharides, as well as other molecules such as proteins. The extracted polysaccharides from these exudates are thermally stable and have good solubility in aqueous media unlike the crude exudate. It would therefore be possible to investigate the potential of these exudates and their polysaccharides source of biosourced polymer materials.

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