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## **Sextonia rubra (Mez.) van der Werff sawmills residues as a valuable resource for the production of larvicidal extracts against *Ae. aegypti* Linnaeus (Diptera: Culicidae)**

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1 ***Sextonia rubra* (Mez.) van der Werff sawmills residues as a valuable resource for the**  
2 **production of larvicidal extracts against *Ae. aegypti* Linnaeus (Diptera: Culicidae)**

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18 Highlights

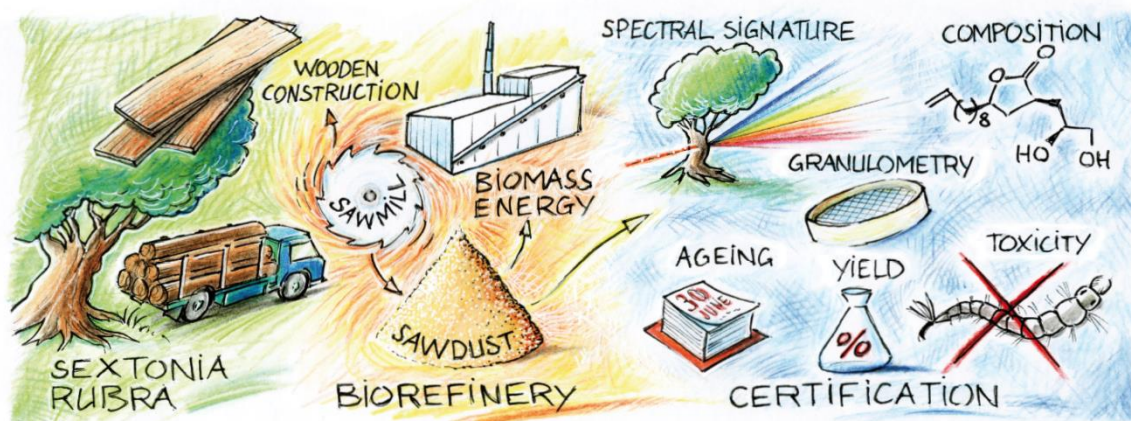
- 19 • *S. rubra* sawdust is a local and homogenous resource promising for valorization  
20 • Rubrenolide/rubrymolide content correlates with *Ae. aegypti* larval mortality  
21 • *S. rubra* sawdust was stored for up to 10 months without loss of its bioactivity  
22 • The crude extract of *S. rubra* shows larvicidal activity after 9 months of storage

23

## 24 Abstract

25 In the development of botanical insecticides, the availability of a certifiable raw material and  
26 the guarantee of a stable biological efficacy over time are crucial factors. *Sextonia rubra* is one  
27 of the main tree species exploited by the Guianese forestry industry, and the valorization of  
28 the large volumes of sawmills residues available would represent an opportunity for the  
29 sustainable development of bioactive products in a biorefinery perspective. In this work, we  
30 studied the variability of the quality of *S. rubra* sawdust according to its origin and investigated  
31 the bioactivity of its extracts against *Ae. aegypti* mosquitoes. We evidenced homogeneous  
32 physico-chemical properties (particle size and NIRS profiles) of *S. rubra* sawdusts  
33 independently of the sawmill of origin, thus highlighting the consistency of the raw material  
34 before extraction. The amount of the  $\gamma$ -lactones rubrenolide and rubrynolide was measured in  
35 12 extracts of *S. rubra* by quantitative NMR (qNMR). GLM regression indicated significant  
36 correlation between these values and larval mortality (adj-R<sup>2</sup> 0.93) for the extracts tested at  
37 30  $\mu\text{g/ml}$ . Additionally, a 48-h DL<sub>50</sub> of 18.6  $\mu\text{g/ml}$  was obtained for a *S. rubra* reference extract.  
38 While sawdust underwent chemical changes during the 10 months of storage, as shown by  
39 NIRS and qNMR analyses, we observed no significant impact on larval mortality for the  
40 extracts tested at 30  $\mu\text{g/ml}$ . A stability of 9-months was also observed for a crude extract stored  
41 at room temperature. Sawmill residues of *S. rubra* thus appear to be suitable raw material for  
42 the production of significant quantities of wood extracts containing larvicidal compounds  
43 against *Ae. aegypti*. Their stability over time also makes it possible to consider production  
44 routes that could be transferred to an industrial scale.

45



47

48 Keywords

49 Biorefinery, circular economy, botanical insecticides, standardization, qNMR, NIRS

50

51 1. Introduction

52 Dengue, Zika, chikungunya and yellow fever are dramatically emerging or re-emerging  
 53 mosquito-borne diseases, and vector control remains a key component in interrupting virus  
 54 transmission by *Aedes* species such as *Ae. aegypti* (Benelli, 2015; Benelli & Mehlhorn, 2016;  
 55 Jones et al., 2021). This issue is particularly important in French Guiana, in a context of strong  
 56 resistances to available vector control products (de Thoisy et al., 2021; Epelboin et al., 2018).  
 57 In order to develop new control solutions against *Ae. aegypti*, natural products from plants  
 58 represent a promising field of application, while questioning about the loss of efficacy,  
 59 unintended effects, and pollution caused by some conventional insecticides (Benelli, 2020;  
 60 Pavela et al., 2019; Silvério et al., 2020). Thus, research on botanical insecticides has seen a  
 61 resurgence in recent years, as these products are considered to combine biological efficacy,  
 62 low resistance development, and human and ecotoxicological safety (George et al., 2014;  
 63 Gerwick & Sparks, 2014; Isman, 2015; Turchen et al., 2020). However, when a promising  
 64 biological activity is identified, some crucial factors must be considered in order to move a step

65 further, and to upscale the encouraging results obtained in the laboratory. Several aspects  
66 must be anticipated to overcome the technical, economic and regulatory limitations required  
67 to develop plant-based insecticides (Isman, 2020; Miresmailli & Isman, 2014). Scalability, in  
68 particular, depends on the availability of the raw material to be extracted. One alternative to  
69 obtain sufficient plant resource for product development is to capitalize on the large quantities  
70 of residues and by-products generated by agro-industrial or timber activities. This sustainable  
71 approach reduces the environmental impact of industrial activities, and promote economic  
72 benefit through recycling and diversification of activities (Borges et al., 2019; de Carvalho et  
73 al., 2021). Biorefinery is being developed in particular for the production of high value-added  
74 products from wood or bark, including extracts, as part of a circular economy (Hasegawa et  
75 al., 2016; Khan et al., 2014; Royer et al., 2012; Verkasalo et al., 2021). But if these concepts  
76 are well known and developed in temperate forests, they are still little considered in tropical  
77 regions. The exceptional level of biodiversity of the Amazonian rainforest, which could be  
78 considered as a guarantee of discovery, is counterbalanced by its great heterogeneity, which  
79 complicates its valorization. As a result, only a few species have been characterized regarding  
80 the quantity and quality of their extractives content in French Guiana, and the larvicidal activity  
81 of wood extracts against mosquitoes is a little studied property. For these reasons, the  
82 development of bioinsecticide products from the residual resource of tropical woods and bark  
83 is an innovative approach.

84 Such strategy could be particularly interesting in French Guiana. This French overseas territory  
85 is almost entirely covered by the Amazonian rainforest, and the timber industry is conducted  
86 there with a marked effort, carried out by both scientists and managers, to ensure sustainable  
87 exploitation (Ballère et al., 2021; CRFB, 2019; Hiltner et al., 2018; Pioniot et al., 2018). In  
88 particular, selective logging by the forestry system generates significant volumes of wood  
89 residues, currently on the order of 30-50,000 t/year for sawmill residues alone (DAAF, 2016).  
90 These co-products are therefore available and abundant resources of interest that could be  
91 valorized.

92 Among locally available wood species, *Sextonia rubra* (Mez.) Van der Werff (Lauraceae)  
93 appears to be a promising resource to achieve this goal. *S. rubra* is a tropical tree species  
94 found in the Guyana shield (French Guiana, Suriname, Guyana and Venezuela) and in the  
95 Brazilian Amazon (Van der Werff, 1997). It is characterized by a remarkably durable  
96 heartwood, naturally resistant to decay, and thus exploited for carpentry, residential  
97 construction, and outdoor applications (CTFT, 1984; Detienne et al., 1989). *S. rubra* heartwood  
98 is known to possess two characteristic  $\gamma$ -lactones compounds, the alkene-alkyne pair  
99 rubrenolide and rubrymolide respectively (Franca et al., 1977; Fu et al., 2018, 2019). These  
100 extractives exhibit biological activities, notably against lignivorous fungi and termites, and  
101 therefore relate to the natural durability of *S. rubra* wood (Houël et al., 2017; Rodrigues et al.,  
102 2011). The remarkable larvicidal activities against *Ae. aegypti* of *S. rubra* extract, rubrenolide  
103 and rubrymolide have also been highlighted previously by members of our group (Falkowski et  
104 al., 2016a; 2020). Given this last result, a patent was filed to promote the valorization of this  
105 extract in the field of vector control (Falkowski et al., 2016b).

106 However, like any natural product, the qualitative and quantitative composition of these  
107 compounds varies on an interspecific and intraspecific scale, depending on tree individuals  
108 and tissues (stem, roots, branches, sapwood and heartwood), as well as on growth-defense  
109 trade-offs or genetic background (Chemetova et al., 2020; Hasegawa et al., 2016; Padovan  
110 et al., 2013; Verkasalo et al., 2021). In addition, wood compounds are also influenced by other  
111 exogenous factors such as humidity, light, or temperature during storage of the raw material  
112 and of the extract (Cabalova et al., 2021; Günther et al., 2021; Kanis et al., 2013). For these  
113 reasons, it is of crucial importance to identify the conditions that allow the production of  
114 standardized extracts of sustained quality in order to valorize these bioactive molecules.

115 With the objective to meet some of the requirements for moving from the discovery of promising  
116 larvicidal wood extracts to the eventual development of products against *Ae. aegypti* larvae, we  
117 therefore embarked upon identifying the factors influencing the quality of *S. rubra* sawdusts  
118 and extracts, and consequently the mortality of *Ae. aegypti* larvae when exposed to wood

119 extracts. To achieve this objective, we first characterized the physico-chemical properties of  
120 the sawmills residues in relation to the sawmill of origin using particle size measurements, and  
121 by analyzing NIRS profiles of the sawdusts. We then investigated the relationship between the  
122 content of the bioactive  $\gamma$ -lactones rubrenolide and rubrynolide in the extracts, assessed by  
123 quantitative NMR, and the larvicidal efficacy of the extracts. Finally, we focused our interest on  
124 studying of the stability of the sawdust and extract over storage time, and its impact on  
125 biological efficacy. This information allowed to discuss the feasibility of an industrial  
126 valorization of *S. rubra* sawdusts for biorefinery in French Guiana.

127

## 128 2. Materials and methods

### 129 2.1. Plant material

130 In French Guiana, the annual volume of logs harvested on the permanent forest area (DFP, or  
131 “Domaine Forestier Permanent”), managed by the National Forestry Office (ONF, Office  
132 National des Forêts) fluctuates between 60,000 and 95,000 m<sup>3</sup> depending on climatic and  
133 logistical conditions (Soulères, 2021). Out of 1700 identified tree species, only about 60 are of  
134 commercial interest, among which a limited number (Angélique, Gonfolos and Grignon franc,  
135 respectively *Dicorynia guianensis* Amshoff, Fabaceae, *Qualea rosea* Aubl. and *Q. albiflora*  
136 Warm., Vochysiaceae and *Sextonia rubra* (Mez.) Van der Werff, Lauraceae) represent 73.9%  
137 of the overall volume harvested over the period 2010-2021. In particular for this same period,  
138 the volume exploited of *S. rubra* represented about 7.5% of the total volume of logs exploited  
139 in French Guiana, i.e. an average value of 5,348 m<sup>3</sup>/year. Sawmill residues of *S. rubra*  
140 (samples of ca. 30 L) were collected in different locations for the study as a co-product of the  
141 primary lumber industry directly during the processing of fresh log, from the residue stream  
142 (Figure 1).

143

144 **Figure 1:** Logging and wood processing in French Guiana: **(a)** tree cutting; **(b)** log yards near  
145 the logging site; **(c)** and **(d)** processing of logs and sawmilling residues (wood sawdust).



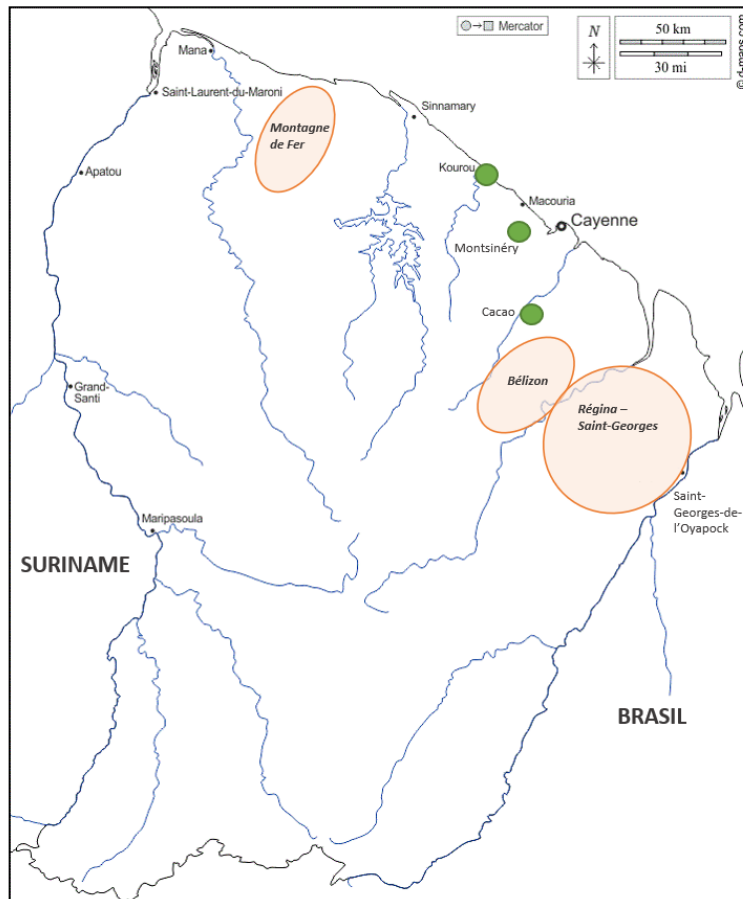
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147

148 Commercial sawmills residues of *S. rubra* used in this study were collected in Montsinéry  
149 (Scierie de Montsinéry, 4°58'34"N 52°26'45"W; September 2017), Cacao (Bois et Sciages de  
150 Guyane, 4°54'31"N 52°19'59"W; February 2019), and Kourou (Scierie du Dégrad Saramaka,  
151 5°08'54"N 52°41'20"W; March 2020) respectively. These sawmills correspond to the most  
152 important suppliers in French Guiana in terms of volumes of sawn wood, and are therefore  
153 representative of the resource that can be obtained for further valorization in this territory  
154 (CRFB, 2019). Notably, the Cacao sawmill processed 38.5% of the total *S. rubra* wood  
155 harvested in 2020 and 2021, and the Kourou sawmill 12.7%. Logs originated from the Bélizon  
156 forest (Régina, 4°16'48"N 52°25'12"W) in the case of the Montsinéry sawmill, from the Régina-  
157 Saint-Georges forest (Mataroni area, Régina, 4°16'15"N 52°09'56"W), and from the Montagne  
158 de Fer (Mana, 5°24'00"N, 53°35'24"W) in the case of the Kourou sawmill (Figure 2).

159

160 **Figure 2:** Distribution of collection sites for sawdust samples of *S. rubra* (French Guiana). The  
161 green dots correspond to the 3 sawmills locations: from West to East: Kourou, Montsinéry and  
162 Cacao. The orange areas indicate the forest areas where the logs originate. The background  
163 map was obtained from d-maps.com, and modified as necessary.



164

165

166 For this study, the acronyms for the commercial sawmill residues samples (SrM, SrC and SrK)  
 167 were created from the combination the name of the wood species (*Sextonia rubra*, abbreviated  
 168 as Sr), and the initial of each location, i.e. M for Montsinéry, K for Kourou, and C for Cacao.  
 169 For sawmill residues SrM, a single batch was collected, while for sawmill residues SrC and  
 170 SrK, 3 batches (SrC\_B1, SrC\_B2, SrC\_B3 and SrK\_B1, SrK\_B2 and SrK\_B3, respectively)  
 171 were obtained, with the three batches corresponding to three successive days of *S. rubra*  
 172 sawing.

173 Together with the commercial sawmill residues, two supplementary samples were added to  
 174 the collection. The aim was to provide additional wood extracts to increase the dataset used  
 175 to study the correlation between the chemical composition of *S. rubra* extracts and their  
 176 larvicidal activity. The first sample was obtained from a commercial wooden plank ground into  
 177 powder prior to extraction in the wood science laboratory of the EcoFoG research unit in

178 Pariacabo, Kourou, leading to the SrP sample. The second sample was obtained by collecting  
179 sawdust directly from a tree cutting in the Bélizon forest (Regina, 4°16'48"N 52°25'12"W, thus  
180 leading to SrR sample), as presented in (Rodrigues et al., 2011), while accompanying the  
181 logger into the forest. All samples were dried at room temperature (21°C, 65% relative  
182 humidity) until equilibrium humidity ( $H < 13\%$ ). The complete sample design is available as  
183 Supplementary Material (Figure S1).

184

## 185 2.2. *S. rubra* sawdust fractioning

186 In order to evaluate the effect of sawmill provenance on the physico-chemical properties of  
187 sawdust, the proportion of three granulometric fractions ( $> 500 \mu\text{m}$ ,  $500\text{-}200 \mu\text{m}$ , and  $< 200$   
188  $\mu\text{m}$  mesh size) was measured for the three wastes from different sawmills (Montsinéry: SrM,  
189 one batch; Cacao: SrC, 3 batches; Kourou: SrK, 3 batches). The particle size fractions were  
190 chosen based on data from the literature dealing with wood valorization in a biorefinery context  
191 (Hasegawa et al., 2016; Karinkanta et al., 2018; Wijayanto et al., 2015). After sieving, the  
192 mass retained on each sieve was weighted and the corresponding mass fraction yields were  
193 determined. For each batch from each sawmill, three 100 g replicate samples were used for  
194 fractioning, and the mean value calculated for each sawmill.

195

## 196 2.3. NIRS spectra acquisition and data processing

197 Near-infrared spectroscopy (NIRS) is an analytical technique which relies on the light  
198 absorption properties chromophore in the near infrared range. These chromophores are  
199 notably found among the hydrogen-containing functional groups of organic compounds, and  
200 the observation of the molecular vibrations of these groups constitute the NIR spectra  
201 (Schimleck et al., 2001; Tsuchikawa and Kobori, 2015). In our case, NIR spectra of sawdust  
202 were used to compare the molecular profiles of sawmill residues from 1/ various geographical  
203 origins and 2/ increasing storage times in controlled conditions (light, humidity, and  
204 temperature) (see 2.7). For the provenance study, the sample design was identical to that used

205 for particle size analysis, with 3 sawdust replicates (10 g) for each commercial sample from  
206 SrM (one batch), SrC (three batches) and SrK (three batches), i.e. 21 samples (3 replicates  
207 for each of the seven batches). For the stability analysis, 3 replicates for each of the 11 storage  
208 times, leading to 33 samples, were used. In each case, the wood powder was ground with a  
209 mill (Retsch ZM 200) using a 500  $\mu\text{m}$  mesh, and analyzed before any extraction process.  
210 Spectra were measured by diffuse reflectance with a Fourier transform spectrometer (MPA,  
211 Bruker Optics, Ettlingen, Germany). The spectral acquisition was performed using the NIR  
212 region from  $12500\text{ cm}^{-1}$  to  $3700\text{ cm}^{-1}$  (800-2700 nm), with a spectral resolution of  $8\text{ cm}^{-1}$ . Each  
213 spectrum was obtained by 32 scans which were averaged to improve the signal-to noise ratio.  
214 Three independent acquisitions were performed for each biological replicate. The laboratory  
215 temperature and relative humidity were kept constant ( $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and  $65\% \pm 5\%$ , respectively)  
216 throughout NIR processing to maintain the samples at 12% theoretical moisture yield. The  
217 collected spectra were analyzed using the Unscrambler software package (version 9.8 Camo  
218 AS, Oslo, Norway).

219

#### 220 2.4. Solvent extraction

221 The wood powder was ground in a mill (Retsch ZM 200) to select a particle size  $\leq 500\text{ }\mu\text{m}$   
222 before extraction. The raw materials were then extracted using ethyl acetate (EtOAc, RPE  
223 grade, Carlo Erba) in a 1:20 ratio (w/v) (10 g of wood powder for 200 mL EtOAc) at room  
224 temperature by maceration during 24h under stirring. After extraction, the solution was filtered  
225 and the solvent removed by evaporation under reduced pressure with a SpeedVac™  
226 concentrator (Savant SPD121P, Thermo Scientific). The resulting crude extracts were stored  
227 in a freezer at  $-18^{\circ}\text{C}$  until assay. To evaluate the impact of the extracts chemistry on larvicidal  
228 activity, a large set of extracts obtained from the different commercial sawmill residues were  
229 used (Figure S1). Extracts SrM1, SrM2 and SrM3 on the one hand, and SrK1 to SrK4 on the  
230 other hand were obtained by extracting sawdust samples from SrM and SrK at different storage  
231 times (2017, 2018 and 2020 for SrM, collected in September 2017, and between April 2020

232 and July 2020 for SrK, collected in March 2020). SrC, SrP and SrR each resulted in a single  
233 extract. As we tried to obtain the widest range of larvicidal mortalities, ideally from 0 to 100%,  
234 fractions containing only the larvicidal compounds rubrenolide and rubrenolide were added to  
235 the dataset. For this purpose, the SrP and SrR crude extracts were partitioned into hexane and  
236 ethyl acetate fractions (RPE grade solvents, Carlo Erba) following the previously published  
237 procedure (Rodrigues et al., 2010). Solvent evaporation of the ethyl acetate fractions led to  
238 SrP+ and SrR+ extracts, respectively, containing only the two  $\gamma$ -lactones.

239

## 240 2.5. Quantitative NMR analysis

241 To perform an accurate analysis of the rubrenolide and rubrymolide content of the extracts,  
242 quantitative Nuclear Magnetic Resonance (qNMR) was used. NMR spectra ( $^1\text{H}$ ,  $^{13}\text{C}$  and 2D  
243 sequences) were recorded on a Varian 400 NMR spectrometer equipped with a 5 mm inverse  
244 probe (Auto X PGF  $^1\text{H}/^{15}\text{N}-^{13}\text{C}$ ). The  $^1\text{H}$  NMR spectra were recorded at 400 MHz and  $^{13}\text{C}$  NMR  
245 spectra at 100.6 MHz. Chemical shifts are expressed in ppm downfield of tetramethylsilane  
246 (TMS), and coupling constants ( $J$ ) are expressed in Hz (s stands for singlet, d for doublet, t for  
247 triplet, q for quartet, m for multiplet, br for broad).

248 Quantitative NMR (qNMR) analyses were performed by adapting a previously published  
249 procedure applied to *S. rubra* wood extracts (Fu et al., 2019). Wood extracts (9-15 mg) and  
250 the quantitative NMR standard (TraceCERT®) 1,2,4,5-tetrachloro-3-nitrobenzene (1,2,4,5-  
251 TCNB, CAS: 117-18-0, Sigma-Aldrich ref.7, 2.5 mg) were dissolved in deuterated chloroform  
252 ( $\text{CDCl}_3$ , 500  $\mu\text{L}$ , Aldrich) in a 2 mL vial before transferring the solution into a 5 mm NMR tube.  
253 1,2,4,5-TCNB was chosen as a standard because (1) its resonance signal does not overlap  
254 with the signals of *S. rubra* extracts, (2) it dissolves readily in  $\text{CDCl}_3$ , and (3) no chemical  
255 reaction occurs between 1,2,4,5-TCNB and the *S. rubra* constituents, as recommended by (Liu  
256 et al., 2020). To ensure sufficient relaxation delay (D1) for accurate quantitative analysis, the  
257 relaxation time ( $T_1$ ) of the standard and rubrenolide were checked using the T1-MEASURE  
258 sequence of the OpenVnmrJ software before any analysis. The NMR acquisition time was set

259 at 2.55 s with a spectral width of 6410.3 Hz and 16 scans were recorded with a relaxation delay  
260  $D1 > 5 \times T1$  (45 s) for a 45° pulse sequence. The  $^1\text{H}$  spectra were phased and baseline  
261 corrected before processing with the ACD/NMR Processor software.

262 For the quantification of rubrenolide and rubrymolide, the  $^1\text{H}$  signal corresponding to the proton  
263  $\delta$  5.81 ppm ; ddt, 1H Re,  $J = 17.0 ; 10.1 ; 6.7$  Hz on the one hand, and the  $^1\text{H}$  signal  
264 corresponding to the protons  $\delta$  4.43 ppm ; ddt, 1H Re and 1H Ry,  $J = 10.8 ; 7.1 ; 5.3$  Hz on  
265 the other hand, were integrated manually. These signals were chosen because they were  
266 clearly separated from the other signals and did not overlap with any other signal regardless  
267 of the extract when 2D spectra were analyzed.

268 The following formula was used for the calculation (Eq. 1):

$$269 \quad m_c = (I_c / I_{\text{std}}) * (N_{\text{std}} / N_c) * n_{\text{std}} * M_c$$

270 Where  $m_c$  is the mass of the compound (Re or Ry),  $I_c$  and  $I_{\text{std}}$  correspond to the value of the  
271 integral signal area of the compounds and 1,2,4,5-TCNB, respectively,  $N_{\text{std}}$  and  $N_c$  are the  
272 number of  $^1\text{H}$  atoms belonging separately to the respective molecular unit of the compounds  
273 and 1,2,4,5-TCNB,  $n_{\text{std}}$  is the molar amount of 1,2,4,5-TCNB and  $M_c$  is the molecular weight of  
274 the compound under consideration (Re or Ry, with  $M_{\text{Re}} = 298.4$  g/mol and  $M_{\text{Ry}} = 296.4$  g/mol).  
275 For the analysis of the relationship between chemical composition and larvicidal effect, each  
276 available extract as described above was sub-sampled in two NMR tubes to provide two  
277 replicates for each analysis, except for SrP and SrK1 for which two independent extractions  
278 were performed. The mean value of the measured areas was used for the calculation. For all  
279 other analyses, each of the three extraction replicates was analyzed once, and the mean value  
280 of the three measurements was used for the calculation. Full details of the calculation are  
281 available in the corresponding tables in the Supplementary Material.

282

283           2.6.    Evaluation of larvicidal activity

284    Insect collection and rearing, larvicidal assay, and data analysis were performed as previously  
285    described (Falkowski et al., 2020). A natural population of *Ae. aegypti* (Diptera: Culicidae)  
286    collected from the city of Cayenne (CAY) was used to test the extracts and compounds. The  
287    choice of this strain was made to obtain results as close as possible to the current context of  
288    French Guiana where *Ae. aegypti* populations exhibit a strong resistance to pyrethroids and  
289    organophosphate insecticides (Guidez et al., 2020). Late third or early fourth-instar larvae from  
290    a first generation (F1) reared from wild caught larvae (F0) were used in the tests. The larvicidal  
291    activity of the extracts was studied using the WHO procedure for testing of mosquito larvicides  
292    (World Health Organisation, 2005). Each bioassay exposed a total of 200 larvae, divided into  
293    eight replicates of 25 larvae, to 1 mL of ethanolic compound solution resuspended in 99 mL of  
294    distilled water to a final concentration of 30 µg/ml. For each test, negative control cups  
295    containing only 1 mL of ethanol in water were run in parallel. The larval bioassays were  
296    subjected to a validation step. First, all tests with a natural mortality in the control greater than  
297    20% were excluded. If mortality in the control was between 5 and 20%, the application of  
298    Abbott's formula (Abott, 1925) was used to correct mortalities in the treated cups. To assess  
299    the larvicidal effect of rubrenolide and rubrenolide compounds, mortalities in treated cups were  
300    compared to the corresponding untreated (control) data using the non parametric Kruskal-  
301    Wallis test. For calculation of LD<sub>50</sub> and LD<sub>90</sub>, the concentrations were adjusted to give 0% to  
302    100% mortality (3 to 120 µg/ml). The SrK4 extract, which was used for the LD<sub>50</sub> and LD<sub>90</sub>  
303    calculations, was obtained from SrK\_B3 sawdust (Kourou sawmill, batch 3), collected in March  
304    2020 and extracted in July 2020.

305

306           2.7.    Chemical and biological stability of *S. rubra* sawdust during storage in  
307                            controlled conditions (light, humidity, temperature)

308    For this study, sampling was restricted to one sawmill, and 100 g of sawdust batch #3 collected  
309    at the Kourou sawmill in March 2020 (SrK\_B3) was sampled each month for 10 months in the

310 conditioning room (21°C, 65 % relative humidity, in the dark). Eleven samples were thus  
311 obtained from March (SrK\_B3\_t0) to December 2020 (SrK\_B3\_t10). The sawdust samples  
312 were then ground with a mill (Retsch ZM 200) to a particle size  $\leq 500 \mu\text{m}$ , and kept at -18°C in  
313 watertight bags until further study to avoid molecular degradation. Once the whole collection  
314 was completed, the eleven samples were extracted as described above, and subjected to  
315 NIRS and qNMR analysis and larvicidal bioassays (30  $\mu\text{g}/\text{mL}$ ) for chemical and biological  
316 evaluations following the above-described protocols. For the calculation of extraction yield, the  
317 moisture content of the sawdust samples was measured and taken into account. For this  
318 purpose, 1.0 g of grated wood was weighted and placed in an oven at 103°C for 24h to  
319 measure the wood moisture content (WMC) in conjunction with the extraction procedure. The  
320 moisture content was calculated according to Eq. 2:

$$321 \text{ WMC} = [(\text{wet wood mass} - \text{dry wood mass}) * 100] / \text{dry wood mass}$$

322 The extraction yield was then calculated using the theoretical anhydrous mass  $m_0$  obtained  
323 according to Eq. 3:

$$324 m_0 = (m_s * 100) / (\text{WMC} + 100)$$

325 where  $m_s$  is the mass of the sawdust sample used for extraction and WMC is expressed as a  
326 percentage. Wood moisture content and extraction yield were obtained as mean values from  
327 three replicate experiments.

328

329 2.8. Stability of the larvicidal activity of the *S. rubra* extracts over a storage  
330 period at room temperature

331 *S. rubra* EtOAc wood extract SrK4, prepared in July 2020 from SrK\_B3 wood powder ground  
332 with a mill (Retsch ZM 200) to a particle size  $\leq 500 \mu\text{m}$  was maintained in an amber flask at  
333 room temperature (25°C) until April 2021 to determine the stability of SrK extract. Larvicidal  
334 activity was determined at intervals of 0, 30, 60, 90, 120, 150, 180 and 280 days after

335 preparation of the extract using a freshly prepared ethanol solution and as described above.  
336 This procedure was adapted from (Kanis et al., 2013).

337

## 338 2.9. Statistical analyses

339 Kruskal-Wallis and pairwise comparisons were performed using the rstatix package of R  
340 software, version 4.1.3 (<https://www.r-project.org/>). PCA analysis was computed using the  
341 factoMineR and factoextra packages. The Bioconductor ropls package was used for PCA  
342 validation and OPLS analysis (<https://bioconductor.org/>). The relationship between the  
343 larvicidal activity of the extracts and their content of biomarkers rubrenolide and rubrymolide  
344 was investigated. It is well characterised that mortality against increasing doses of an  
345 insecticide follows a sigmoidal distribution from 0 to 100% of mortality modeled by the probit  
346 function. Mortality data were transformed into percent mortality. The quantity of rubrenolide  
347 and rubrymolide in the extract was expressed as a percentage of the total extract. We therefore  
348 subjected the dataset to a generalized linear model (GLM, family quasibinomial, link = probit)  
349 to validate the relationship between rubrenolide and rubrymolide content and mortality, and to  
350 investigate the effect of individual or combined molecules on larval mortality. All tests were  
351 performed using the MASS and car packages of R software, version 3.2.0 ([https://www.r-](https://www.r-project.org/)  
352 [project.org/](https://www.r-project.org/)). Lethal doses were obtained by log-Probit regression under a general linearized  
353 model using the MASS package in R software version 3.2.0 (<https://www.r-project.org/>) as  
354 previously described (Falkowski et al., 2016a).

355

## 356 3. Results and discussion

### 357 3.1. Influence of sawmill origin on sawdust particle size

358 In order to progress towards an efficient valorization of sawdust and wood residues, the  
359 technological characteristics of the raw resource can be explored to improve the description of  
360 its quality. The first step of our study was therefore to evaluate the effect of sawmill origin on

361 sawdust particle size. In our hands, few coarse particles were visually present in the wood raw  
362 material, and bark fragments were only sporadically present among the wood residues (Figure  
363 3).

364

365 **Figure 3:** (a) *Sextonia rubra* (Mez.) Van der Werff (Lauraceae, « Grignon franc ») in the  
366 Guianese forest of Paracou; (b) Sawdust of *S. rubra* (Sr) collected in the Montsinéry sawmill  
367 (labelled as SrM sample) ; (c) Sawdust fractions obtained after manual removal of any visible  
368 bark material and sieving: particle size > 500  $\mu\text{m}$  (left), particle size 500-200  $\mu\text{m}$  (middle),  
369 particle size < 200  $\mu\text{m}$  (right).



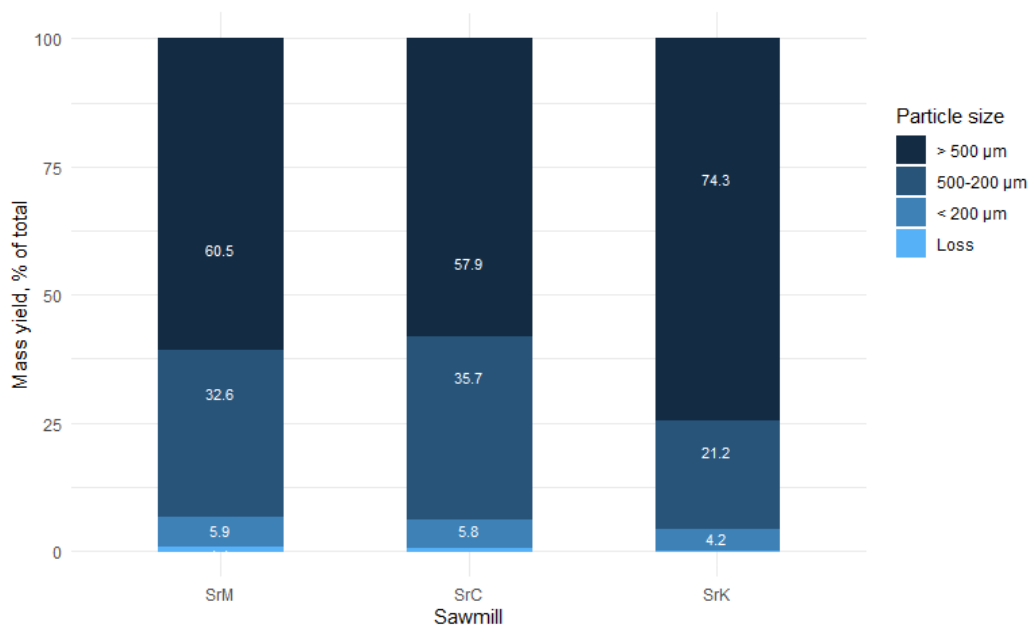
370

371

372 The granulometric fractions obtained from the different sawmills where wood residues were  
373 collected are summarized in Figure 4:

374

375 **Figure 4:** Barplots of mass distribution by particle size after fractioning of commercial *Sextonia*  
 376 *rubra* (Sr) wood wastes from Monstinéry (labelled as SrM samples), Cacao (labelled as SrC  
 377 samples), and Kourou (labelled as SrK samples) sawmills.



378

379 We observed that the three sawmills led to an overall similar profile. For each of the sawmills  
 380 studied, more than 50% (from  $57.9 \pm 19.5\%$  to  $74.3 \pm 2.9\%$ ) of the resource was composed of  
 381 particles characterized by a size  $> 500 \mu\text{m}$ . Particles between 200 and  $500 \mu\text{m}$  in size  
 382 represented  $21.2 \pm 2.1\%$  (SrK) to  $35.7 \pm 16.7\%$  (SrC) of the total amount. Finally, fines  
 383 (size  $< 200 \mu\text{m}$ ) accounted for less than 6% (from  $4.2 \pm 0.6\%$  to  $5.9 \pm 0.2\%$ ) of the total mass  
 384 yield. The notable amount of medium-sized particles provided an effective accessible surface  
 385 area for extraction (Hasegawa et al., 2016; Miranda et al., 2012; Wang & Weller, 2006).  
 386 Moreover, the small amount of very small particles ( $< 200 \mu\text{m}$ ) can notably prevent clogging  
 387 during filtering steps following the industrial scale extraction process (Bostyn et al., 2018). High  
 388 standard deviations and coefficient of variations were, however, obtained for the Cacao  
 389 sawmill (Tables S1 and S2) due to the particularities of one out of three batches (Batch N°1,  
 390 SrC\_B1). This may probably be due to a difference in the process used by the sawmiller to  
 391 recover this particular sawdust. The collection of sawdust at the top or at the bottom of the  
 392 wastes' pile could for example account for the difference in particle size observed between

393 Batch SrC\_B1 ( $84 \pm 6\%$  of particles  $> 500 \mu\text{m}$ , and  $2 \pm 1\%$  of particles  $< 200 \mu\text{m}$ ) and batches  
394 SrC\_B2 and B3 (in average:  $45 \pm 2\%$  of particles  $> 500 \mu\text{m}$ , and  $7 \pm 1\%$  of particles  $< 200 \mu\text{m}$ ).  
395 However, our results highlighted that regardless of the sawmill, the transformation processes  
396 seemed to be globally similar, leading to a homogeneous distribution. The particle size  
397 distribution obtained for commercial wood waste collected in different sawmills in French  
398 Guiana seemed suitable for a process of valorization of the resource by the development of  
399 value-added products without consequent pre-treatment steps. Indeed, as fine grinding of  
400 wood requires increasing energy with decreasing particle size, it is important to directly obtain  
401 a raw material with interesting technological properties for further processing and valorization  
402 (Karinkanta et al., 2018). These data are of interest for sawmillers interested in biorefinery  
403 initiatives, and can for example be considered by those knowledgeable in the technology  
404 datasets on milling routes as proposed by Fabre et al. (2020).

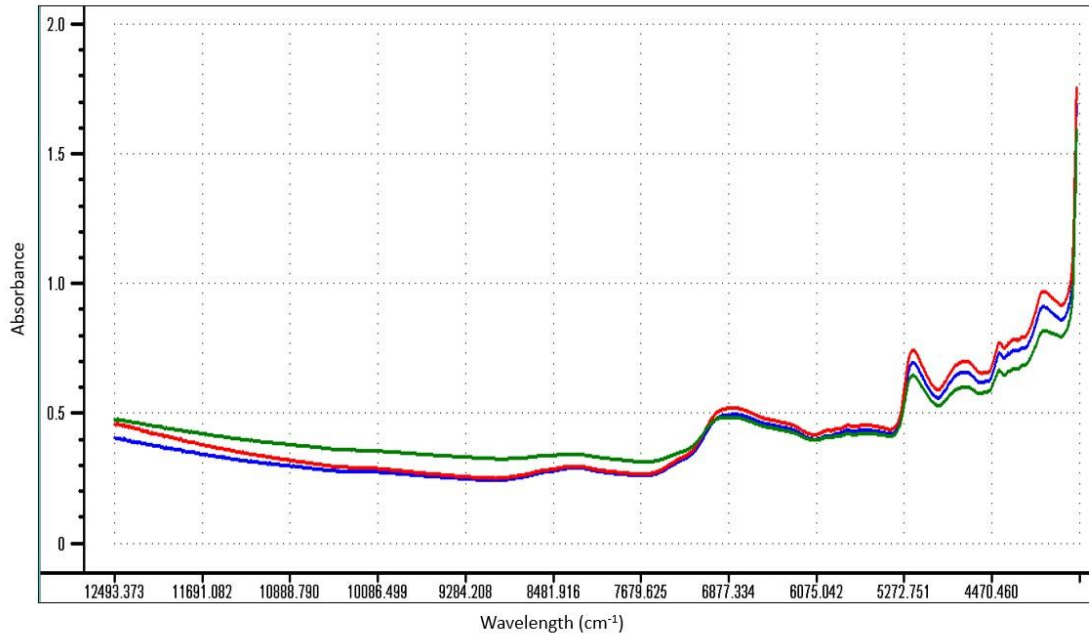
405

### 406 3.2. Influence of sawmill provenance on sawdust NIRS profiles

407 Following this analysis of sawdust physical properties, samples from the three sawmills were  
408 subjected to near-infrared spectroscopy (NIRS) analysis. This rapid, powerful, non-destructive  
409 and cost-effective predictive technique provides information about both the high-weight  
410 chemical groups present in lignin, cellulose, and hemi-cellulose as well as the low-weight  
411 extractives (Tsuchikawa and Kobori, 2015). It can be used both qualitatively and quantitatively  
412 in the forestry industry, for example to predict essential oil or extractive yields and natural  
413 durability, or to discriminate wood samples based on their geographical origin (Alves et al.,  
414 2012; Amusant et al., 2016; Karlinasari et al., 2021; Niamké et al., 2014). It thus provides a  
415 useful tool for tree or wood raw material selection by wood managers (Wang et al., 2022). The  
416 mean pattern of the original NIR spectral data of sawdust samples obtained from the three  
417 sawmills selected in our study (SrM, SrC, SrK) is shown in Figure 5.

418

419 **Figure 5:** Representative average of original near-infrared (NIR) spectra of *S. rubra* (Sr)  
420 sawdust samples collected from the sawmill of Montsinéry (SrM, green), Cacao (SrC, red) and  
421 Kourou (SrK, blue).



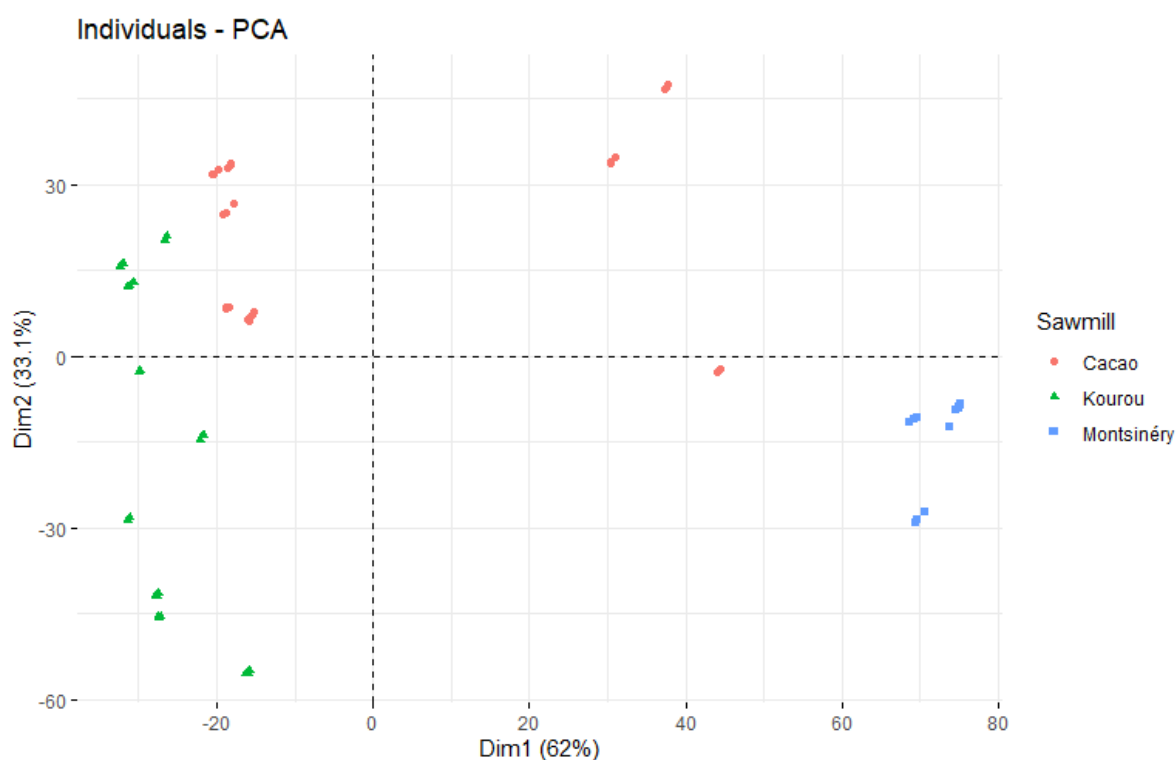
422  
423  
424 The spectra obtained showed an absorbance range between 0.2 and 1.7. The overall profiles  
425 were globally similar, with cellulose, hemicellulose, and lignin being the dominant components  
426 (Schwanninger et al., 2011). Within the acquisition window, two broad absorption bands were  
427 observed, which were associated with water OH-bond ( $7140\text{-}6100\text{ cm}^{-1}$  and  $5360\text{-}4980\text{ cm}^{-1}$ )  
428 (Alves et al., 2012; Büning-Pfaue, 2003; Curcio & Petty, 1951). It appeared that the sawmill of  
429 origin influenced the signal height rather than the overall shape of the NIR spectrum of the  
430 sawdust samples. Spectral differences related to variations in chemical composition were  
431 rather small and, in most cases, invisible by simple visual inspection of the spectra. To better  
432 visualize the differences between the NIR spectra, multivariate statistical analyses were  
433 performed. A PCA was used to represent the NIRS data of *S. rubra* sawdust (Figure 6). The  
434 cumulative  $R^2X$  value was 0.62, and dimensions 1 and 2 accounted for 62 and 33.1% of the  
435 variance, respectively. The OPLS-DA analysis performed on the data according to the sawmill

436 of origin validated the model, with coefficients of determination ( $R^2X$  and  $R^2Y$ ) close to 1 and  
437 revealing a good prediction, and a root mean square error (RMSE) of 0.04 (Figure S2).

438

439 **Figure 6:** PCA (principal component analysis) of NIR spectral data *Sextonia rubra* sawdusts  
440 based on sawmill origin.

441



442

443

444 The results obtained presented several major insights. First, OPLS-DA allowed for the  
445 segregation of spectral data by sawdusts origin, as previously described in the literature  
446 (Karlinsari et al., 2021). However, the proximity of Kourou samples and most of the Cacao  
447 samples did not indicate major chemical differences in these sawdusts, in light of the similarity  
448 observed for the particle size analysis of these samples. The PCA representation also  
449 indicated some disparity in the Cacao sawdust, especially in one out of three batches, as  
450 already observed for the grain size analysis (batch SrC\_B1). Indeed, rubrenolide and  
451 rubrynlolide were found only in specific anatomical structures in *S. rubra* wood, namely ray  
452 parenchyma cells and oil cells (Fu et al., 2018; 2019). As anatomical structures of the wood

453 display various degrees of mechanical resistance during sawing or milling processes, different  
454 fibrous granulometric fractions may thus have different chemical compositions (Vázquez et al.,  
455 2001). This could explain the observed specific disparities between the batches collected in  
456 the Cacao sawmill. However, the similarity observed in the spectral data pattern in Fig. 5  
457 indicated that *S. rubra* remained the processed species, and that this inconsistency did not  
458 come from botanical confusion (Tuncer et al., 2022). Chemistry may explain the singularity of  
459 SrM (*S. rubra* sawdust from the Montsinéry sawmill) samples, which appeared clearly  
460 discriminated from those from Kourou and Cacao. Indeed, in the case of the SrM samples,  
461 collected in 2017 and analyzed in 2020, the sawdust storage process lacked efficacy due to  
462 logistic issues. The higher moisture content of the raw material may have led to biological and  
463 chemical changes. In fact, moisture content has previously been shown to influence the level  
464 of wood biodegradation when exposed to soil microflora, with both parameters being positively  
465 correlated (Amusant et al., 2014; Günther et al., 2021). In addition, endophytic wood fungi are  
466 also known to play a role in wood degradation (Baum et al., 2003; Cline et al., 2018; Leonhardt  
467 et al., 2019). Therefore, an increase in sawdust moisture may have promoted the development  
468 of fungal and bacterial flora, eventually leading to chemical modification through  
469 biotransformation processes (Menelle et al., 2021). The impact on such a process will be  
470 discussed below in the light of the biological activity of the extracts.

471

472 Finally, despite the particular cases discussed above, the sawdust collected in French Guiana  
473 also appeared homogeneous in terms of NIR chemical profiles, especially in two of the most  
474 important sawmills in the territory (Kourou and Cacao). This point is particularly interesting,  
475 because the logs processed in these two sawmills came from two different forest areas, located  
476 respectively in the western part (Montagne de Fer forest, for the Kourou sawmill) and in the  
477 eastern part (Régina - Saint Georges forest, Mataroni area, for Cacao sawmill) of the territory.  
478 Indeed, some similarity between the NIRS data of ebony wood from various geographical  
479 areas of the Celebes Islands has already been observed (Karlinasari et al., 2021).  
480 Furthermore, chemical analysis of extractives in Scots pine sawmill residues showed only

481 small differences regarding the concentration of these compounds in sawdust collected from  
482 four regions from northern to southern Finland (Verkasalo et al., 2021). These results are  
483 therefore encouraging for the production of bioactive extracts from this resource. However,  
484 they highlight that special attention must be paid to the collection and storage steps of sawdust.  
485

### 486 3.3. Chemical composition of *S. rubra* extracts depending on sawdust 487 provenance, age and extraction process

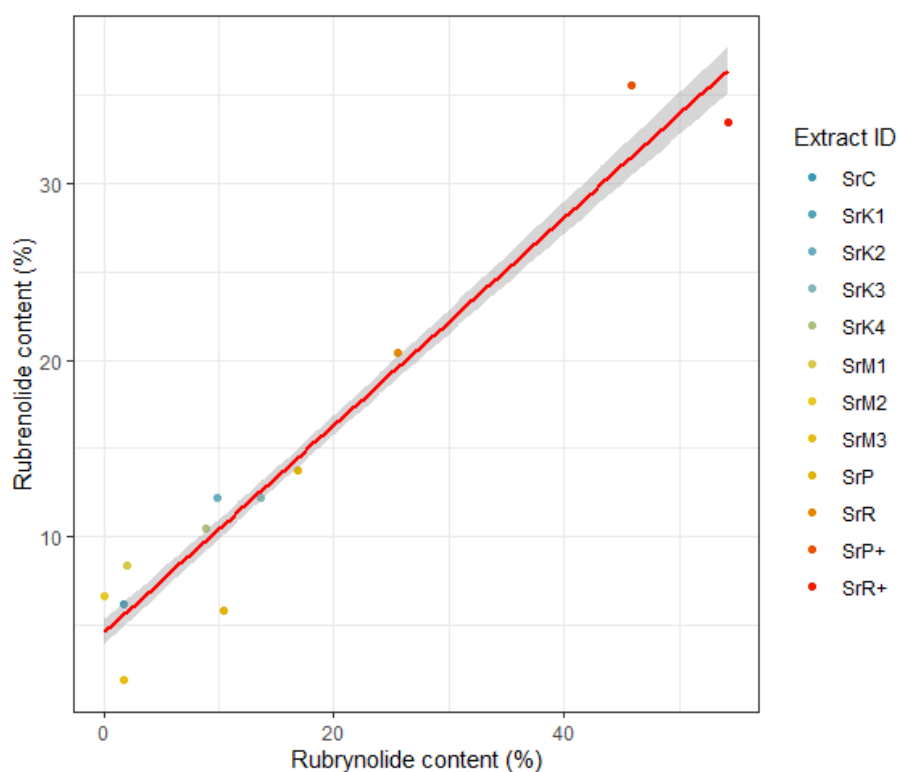
488 The next objective was to better understand the link between the chemistry of the *S. rubra*  
489 sawdust extracts and the larvicidal effect of the extracts against *Ae. aegypti*, complementing  
490 to previous preliminary bioassays (Falkowski et al., 2016a, 2020). With this in mind, NMR  
491 spectra of the *S. rubra* extracts full dataset, as described in the experimental section, clearly  
492 confirmed the presence of both compounds in the extracts. Full details of the calculations and  
493 the dataset obtained for the quantitative NMR analysis are given in Table S3 of the  
494 Supplementary Material. Chemical shifted in agreement with previously published data  
495 (Rodrigues, 2010). In addition to the reference signals at  $\delta$  4.43 and 5.81 ppm, the identification  
496 of the compounds was verified by other characteristic signals, such as the H-14 protons of  
497 rubrenolide ( $\delta$  4.99 ppm,  $J$  = 17.4 Hz, H-14b;  $\delta$  4.93 ppm,  $J$  = 10.2 Hz, H-14a), and protons of  
498 the hydroxylated lateral chain present in both compounds ( $\delta$  3.80 ppm, m, H-2';  $\delta$  3.65 ppm,  $J$   
499 = 10.9, 3.5 Hz, H-3'b;  $\delta$  3.47,  $J$  = 11.0, 6.3 Hz, H-3'a). Finally, protons from the  $\gamma$ -lactone ring,  
500 also present in both compounds, were identified at  $\delta$  2.92 ppm (dtd,  $J$  = 12.1, 7.9, 7.4 Hz, H-  
501 2) and  $\delta$  2.54 ppm (ddd,  $J$  = 13.3, 8.6, 5.5 Hz, H-3). The other signals below 2.5 ppm could not  
502 be distinguished due to the presence of many lipophilic compounds in our extract, showing a  
503 high density of signals in this area (Figure S3). An important point to emphasize is that,  
504 regardless of the origin of the wood (logs or sawmill origin, raw material collection process,  
505 storage time and process), rubrenolide and rubrynlolide were always present in ethyl acetate  
506 extracts. This result is consistent with all previous published studies on the wood chemistry of

507 *S. rubra* (see references cited throughout this article), clearly highlighting these compounds as  
508 chemical markers of this species.

509 Rubrenolide content ranged from 1.9 to 35.5% in the extracts (cutoff values obtained for SrM3  
510 and SrP+ extracts, respectively), with a median value (MV) of 11.4% for all extracts.  
511 Rubrynlolide content ranged from 0.1% (SrM2) to 54.2% (SrR+) with a median value of 10.1%  
512 (Figure 7; Table S4). Interestingly, when observed against the PCA based on NIRS data  
513 (Figure 6), these results supported that the imperfect storage conditions of SrM sawdust  
514 between 2017 and 2020 may have led to some degradation of the chemical content of the  
515 resulting extracts, as discussed above (Günther et al., 2021). Regarding the SrR extract, the  
516 sawdust in this case was collected directly during tree cutting, and not in the log processing  
517 stream. The lack of log storage before sawdust collection could have therefore explained the  
518 high content of rubrenolide and rubrynlolide in this extract. The linear Model ( $R_y \sim R_x$ ) revealed  
519 a linear relationship between the two molecule contents with a significant slope estimate (SE)  
520 of 1.59 (0.04) and a model-adjusted  $R^2$  of 0.93. The Spearman rho correlation reached 0.97  
521 ( $p$ -value =  $7.57e-07$ ). Rubrynlolide was then estimated to be 1.5 times more abundant than  
522 rubrenolide based on that model. Therefore, these two compounds were not only always  
523 present regardless of the extract, but their content varied overall in a linear fashion regardless  
524 of the total amount of these two compounds in a given extract.

525

526 **Figure 7:** Scatter plot of rubrynlolide content versus rubrenolide one and lm regression line  
527 with  $CI_{95}$  interval. The acronyms of the extract IDs are described comprehensively in the  
528 experimental section and Figure S1. Briefly, Sr stands for *S. rubra*, and the following capital  
529 letter for the place of origin (C: Cacao; K: Kourou; M: Montsinéry; P: Pariacabo; R: Régina).  
530 The numbers from 1 to 4 correspond to the extracts at different storage times of the sawdust.  
531 SrP+ and SrR+ correspond to the fractionation of SrP and SrR crude extracts, leading to a  
532 purified mixture of the two  $\gamma$ -lactones rubrenolide and rubrynlolide.



533

534

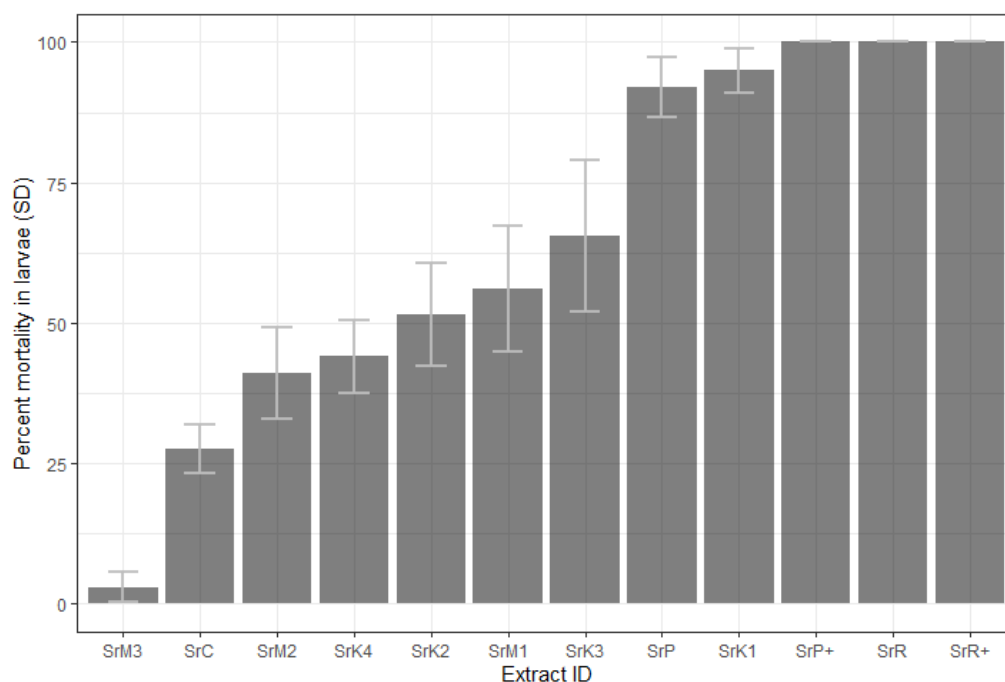
### 535 3.4. Characterization of the larvicidal properties of *S. rubra* extracts

536 The toxic action of 30- $\mu$ g/ml extracts was monitored on the CAY F1 generation. Among the  
 537 12 extracts used, mortality in bioassays varied from 0 ( $3 \pm 3\%$ ) to 100% after 48h of exposure  
 538 (MV: 61%) (Figure 8, full dataset available in Table S5).

539

540 **Figure 8:** Percent mortalities and standard error of the larval test exposing *Aedes aegypti* CAY  
 541 F1 larvae to 30- $\mu$ g/ml *Sextonia rubra* extracts. The acronyms of the extract IDs are described  
 542 comprehensively in the experimental section and Figure S1. Briefly, Sr stands for *S. rubra*, and  
 543 the following capital letter for the place of origin (C: Cacao; K: Kourou; M: Montsinéry; P:  
 544 Pariacabo; R: Régina). The numbers from 1 to 4 correspond to the extracts at different storage  
 545 times of the sawdust. SrP+ and SrR+ correspond to the fractionation of SrP and SrR crude  
 546 extracts, leading to a purified mixture of the two  $\gamma$ -lactones rubrenolide and rubrenolide.

547



548

549

550 SrP+ and SrR+ extracts, as well as SrR extracts, i.e. those with the highest rubrenolide and  
 551 rubrynlolide contents, reached the maximal mortality value in this assay. SrM3 on the contrary,  
 552 obtained from sawdust collected in the sawmill of Montsinéry in 2017 and extracted in 2020  
 553 after imperfect storage conditions, led to the lowest larval mortality. For its part, the SrC extract,  
 554 obtained from the Cacao sawmill and used in this dataset, was indeed obtained from the  
 555 SrC\_B1 outlier batch, discussed in the particle size and NIRS data analyses. In terms of larval  
 556 mortalities, the SrC extract was close to those of SrM, which is consistent with the vicinity  
 557 observed from the Cacao sawmill outlier batch and the Montsinéry samples in the principal  
 558 component analysis of the NIR spectral data. Although the two datasets cannot be exactly  
 559 compared, the results obtained nevertheless show good consistency between NIRS and  
 560 qNMR results.

561 Finally, and to test whether these compounds can be used not only as chemical markers of  
 562 botanical species, but also as biomarkers of the larvicidal activity of extracts obtained from  
 563 sawdust of various origins, the raw composition and mortality data were subjected to statistical  
 564 analysis. Further investigation thus led to run a GLM regression integrating the contents of the

565 molecules as separate variables. Rubrenolide and rubrymolide as single variables, as well as  
 566 in the additive model, were shown to be related with larvicidal activity (Table 1; Pr ( $>|t|$ )  $< 0.05$ ).  
 567 When modelling the interaction of these two compounds, rubrymolide was found to be the only  
 568 molecule with a significant estimate and effect (Pr ( $>|t|$ ) =  $7.07e-07$ , Table 1). Neither  
 569 rubrenolide nor the interaction estimates were significant in the model. Dependency of Re and  
 570 Ry may have explained such results. Nevertheless, both molecules were related to mortality.  
 571 Alongside with the obtained data for these compounds tested individually against the natural  
 572 deltamethrin-resistant Cayenne strain (Table S5), this statistical analysis highlighted that  
 573 rubrenolide and rubrymolide can clearly be considered as biomarkers of larvicidal activity of  
 574 *S. rubra* wood extract, especially in a standardization perspective. Additionally, their potential  
 575 synergistic effect could be investigated to progress towards the comprehension of the  
 576 mechanisms of *S. rubra* wood extract larvicidal activity.

577

578 **Table 1:** Results obtained for the GLM regression

	Estimate	Std. Error	t value	Pr ( $> t $ )
<b>Rubrenolide (Re)</b>				
(Intercept)	-1.15463	0.16660	-6.931	5.20e-10
Re content	0.14392	0.01684	8.544	2.27e-13
<b>Rubrymolide (Ry)</b>				
(Intercept)	-0.677528	0.093263	-7.265	1.08e-10
Ry content	0.108098	0.009888	10.932	2,00E-16
<b>Added</b>				
(Intercept)	-1.004556	0.120070	-8.366	5.41e-13
Summed content	0.069191	0.006534	10.589	2,00E-16
<b>Interaction</b>				
(Intercept)	-0.915351	0.188496	-4.856	4.89e-06
Re content	0.037478	0.027638	1.356	0.178
Ry content	0.112214	0.021069	5.326	7.07e-07
Re:Ry	-0.001488	0.001169	-1.274	0.206

579

580 To complement these data, lethal doses were measured for SrK4 extract, chosen as a  
 581 reference extract because its rubrenolide content ( $10.5 \pm 0.0\%$ ) and rubrymolide content ( $8.9 \pm$

582 0.0%) were close to the median values for the dataset composed of the 12 extracts (11.4 and  
583 10.1% respectively). The LD<sub>50</sub> obtained, measured against the wild type CAY strain, were 39.6  
584 and 18.6 µg/mL at 24h and 48h, respectively, and the LD<sub>90</sub> were 118.2 µg/ml and 51.2 µg/ml  
585 at 24h and 48h, respectively. These data confirmed the promising values measured in a  
586 previous study of *S. rubra* wood extract against the laboratory strain Paea, with a LD<sub>50</sub> value  
587 of 31.7 µg/ml at 48h (Falkowski et al., 2020). These results were also of notable interest when  
588 compared to data in the literature regarding natural products active against *Aedes*. Notably,  
589 species in the genus *Piper* are known to have numerous biological activities (Salehi et al.,  
590 2019). These activities include strong potential in the field of biocontrol, with LC<sub>50</sub> values  
591 ranging from 20 to 65 µg/mL for various *Piper* species, whether plant extracts or essential oils,  
592 against insecticide-susceptible reference laboratory strains, or pyrethroid-resistant wild strains  
593 (Falkowski et al., 2020; Pereira Filho et al., 2021; Lija-Escaline et al., 2015; Scalvenzi et al.,  
594 2019). Interestingly, *P. ovatum* extracts standardized to the active compound piperlongumine  
595 led to LC<sub>50</sub> and LC<sub>90</sub> values of 2.1 and 4.1 µg/ml at 24h respectively, highlighting the value of  
596 correlating the content of bioactive compounds in extracts and larvicidal activity (Kanis et al.,  
597 2013). Other tropical species have also been tested for their larvicidal activities against *Ae.*  
598 *aegypti*, with different endpoint criteria, with LC<sub>50</sub> values below 100 µg/mL being a relevant  
599 reference value (Jian et al., 2022; Mendes et al., 2017), although outstanding values  
600 <10 µg/mL can sometimes be found (Machado Rodrigues et al., 2021; Pavela, 2015; Pavela  
601 et al., 2019). Interestingly, formulations based on natural products (extracts or essential oils)  
602 can achieve remarkable LC<sub>50s</sub> (Faustino et al., 2020). This question will be addressed for  
603 *S. rubra* in a future study, together with the cyto- and eco-toxicity evaluation of both extract  
604 and formulation.

605

606 3.5. Chemical and biological stability of *S. rubra* sawdust and extracts during  
607 storage and effect on larvicidal activity

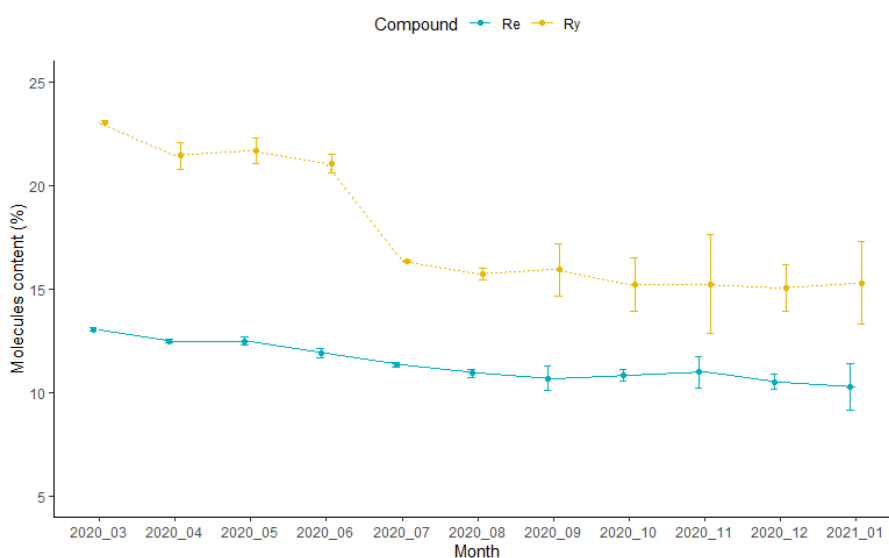
608 Another crucial criterion to be evaluated in the perspective of the commercial development of  
609 a plant-based insecticide is the stability of the product during storage time. While  
610 biodegradation in the environment is presented as an advantage of botanical active products  
611 over synthetic ones, to avoid phytoaccumulation and soil pollution, and to limit unintended  
612 effects, sufficient stability of bioactive compounds is however necessary to ensure long-lasting  
613 efficacy (Kanis et al., 2013). Plant metabolites are indeed sensitive to photodegradation,  
614 hydrolysis, oxidation or evaporation, with loss of biological activity potentially observed when  
615 botanical products are exposed to light, temperature, environmental pH, or microbial activity,  
616 whether in the raw material or the extract (Amer & Mehlhorn, 2006; Isman, 2020; Khan et al.,  
617 2014; Shaalan et al., 2005). Here, we evaluated both the chemical and biological stability of  
618 sawdust and a crude extract over time under controlled storage conditions.

619 The relative humidity of fresh wood residues ranged from  $7.7 \pm 0.5\%$  to  $11.1 \pm 0.3\%$  with an  
620 average value of  $9.4 \pm 1.0\%$ , and thus remained below the 13% limit that ensures good  
621 conservation of the raw material (Table S6). The extraction yield ranged from  $2.6 \pm 0.1\%$  to  
622  $3.0 \pm 0.1\%$ , with an average value of  $2.8 \pm 0.2\%$ , confirming the extraction data discussed  
623 above in this study (Table S7). Rubrenolide and rubrymolide content decreased from  $13.0 \pm$   
624  $0.1\%$  in March 2020 to  $10.3 \pm 1.1\%$  in January 2021, with a mean value of  $11.4 \pm 0.9\%$ , and  
625 from  $23.0 \pm 0.1\%$  in March 2020 to  $15.3 \pm 2.0\%$  in January 2021, with a mean value of  $17.6 \pm$   
626  $3.2\%$ , respectively (Figure 9). This indicated a decrease in the content of both molecules over  
627 a 10-month storage period. Kruskal-Wallis rank sum tests performed on both datasets yielded  
628 a  $p$ -value of 0.005 for rubrenolide content (Chi-squared = 25.2, df = 10) and a  $p$ -value of 0.009  
629 for rubrymolide content (Chi-squared = 23.4, df = 10), indicating a statistically significant change  
630 in the content of both compounds. Notably, a decrease in rubrymolide content seemed to  
631 appear from the 4<sup>th</sup> month of storage (July 2020), while the decline in rubrenolide content was  
632 regular. These results are consistent with the data discussed along the study, notably the

633 elevated amounts in rubrenolide and rubrynolide of the SrR extract, obtained from sawdust  
634 collected directly in the field and without any storage of the log before milling. It also shed light  
635 on previous observations, regarding SrM batches, which encountered imperfect storage  
636 conditions.

637

638 **Figure 9:** Rubrenolide (Re, blue, lower curve) and rubrynolide (Ry, yellow, upper curve)  
639 content (% w/w) and standard error calculated by the qNMR experiment. Wood extracts were  
640 obtained from sawmill residues stored under controlled conditions between March 2020 (t0)  
641 and January 2021 (t10).



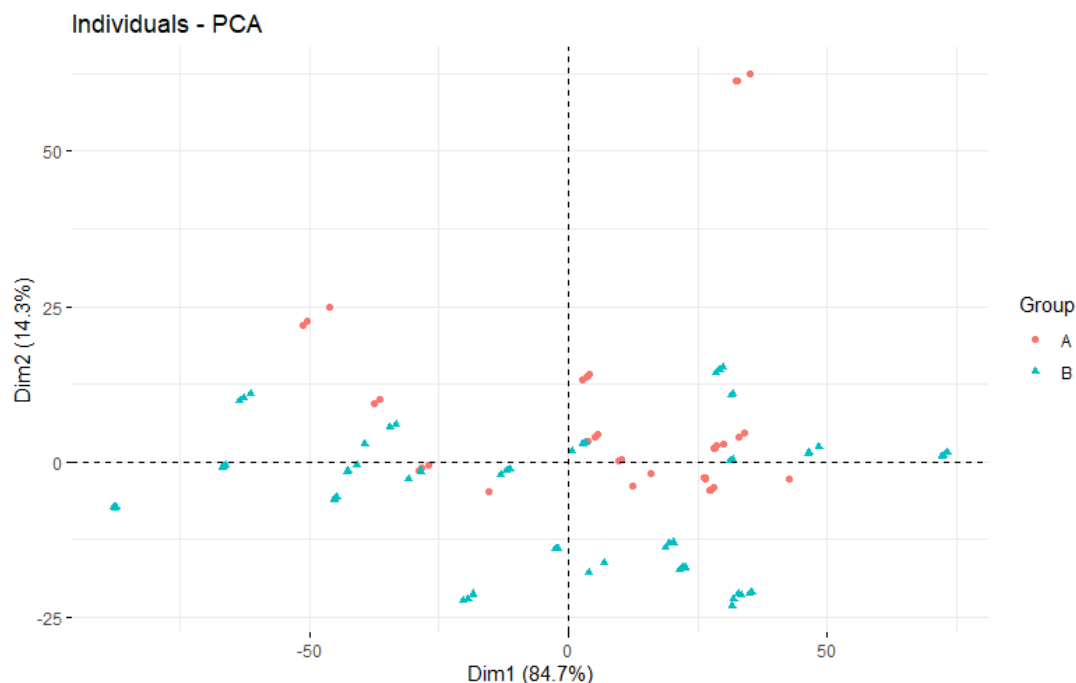
642

643

644 To complement this information, all raw sawdust were subjected to NIRS analysis, to compare  
645 chemical profiles over the storage period, a PCA was performed (Figure 10). The cumulative  
646  $R^2X$  value was 0.85, and dimensions 1 and 2 accounted for 84.7 and 14.3% of the variance,  
647 respectively. To contrast NIRS data with the previous qNMR observation, highlighting a  
648 change in rubrynolide content between samples t0-t4 (March 2020 - June 2020) and t5-t10  
649 (July 2020 - January 2021), data were organized into two groups (A: t0-t4 and B: t5-t10).

650

651 **Figure 10:** PCA (principal component analysis) of NIR spectral data of *Sextonia rubra*  
652 sawdusts as a function of sampling time. Group A: t0-t4 (March 2020 - June 2020); Group B:  
653 t5-t10 (July 2020 - January 2021)



654

655

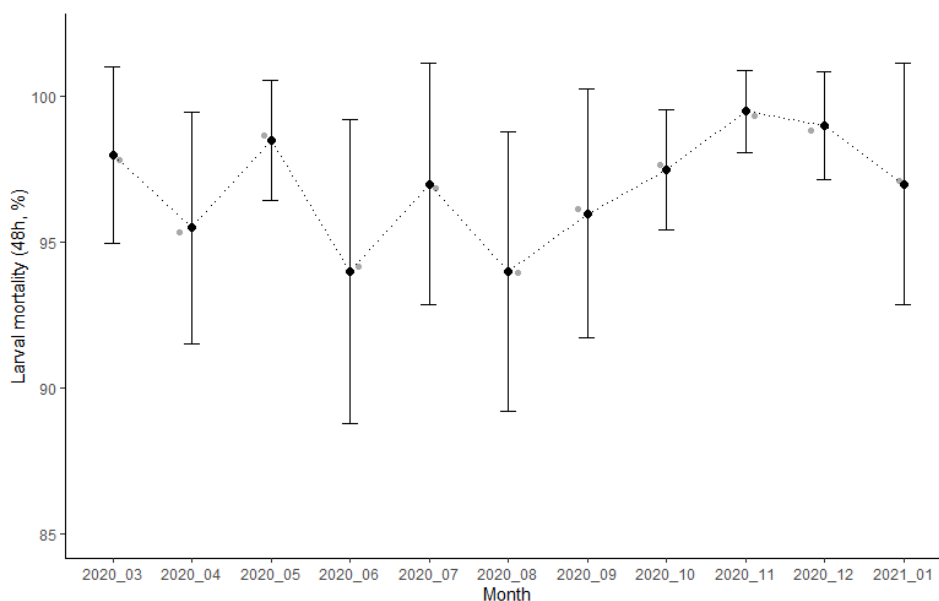
656 In this representation, some overlap between the two groups could be observed. To gain  
657 accuracy, multivariate statistical analysis was used, and OPLS-DA analysis performed on the  
658 data in relation to sampling time. The resulting model showed an  $R^2X$  value of 0.998, a  $R^2Y$   
659 value of 0.891 and a  $Q^2Y$  value of 0.838. The RMSE value was 0.164 (Figure S4), allowing  
660 discrimination between the chemical profile of short-term and long-term storage of *S. rubra*  
661 sawmill residues.

662 The question was then to know if these chemical modifications had a significant impact on  
663 the larvicidal activity of the extracts. Larval mortality after 48h exposure of animals to a 30-  
664  $\mu\text{g/mL}$  solution of *S. rubra* wood extract was therefore evaluated, and the results obtained are  
665 represented in Figure 11, and the full data are available in Table S8. Mortality ranged from  
666  $94 \pm 4\%$  to  $99.5 \pm 1\%$ , with a mean value of  $97 \pm 4\%$ , thus highlighting in all cases a high  
667 biological effect of the extracts on *Ae. aegypti* CAY larvae. The Kruskal-Wallis test yielded a

668  $p$ -value of 0.055 (Chi-squared = 18.0, df = 10), which showed that no significant difference in  
669 larval mortality could be observed as a function of sawdust storage time of the.

670

671 **Figure 11:** Percent mortality and standard error of the larval test exposing *Aedes aegypti* CAY  
672 F1 larvae to 30- $\mu$ g/ml *Sextonia rubra* extracts during a 10-month sawdust storage period.

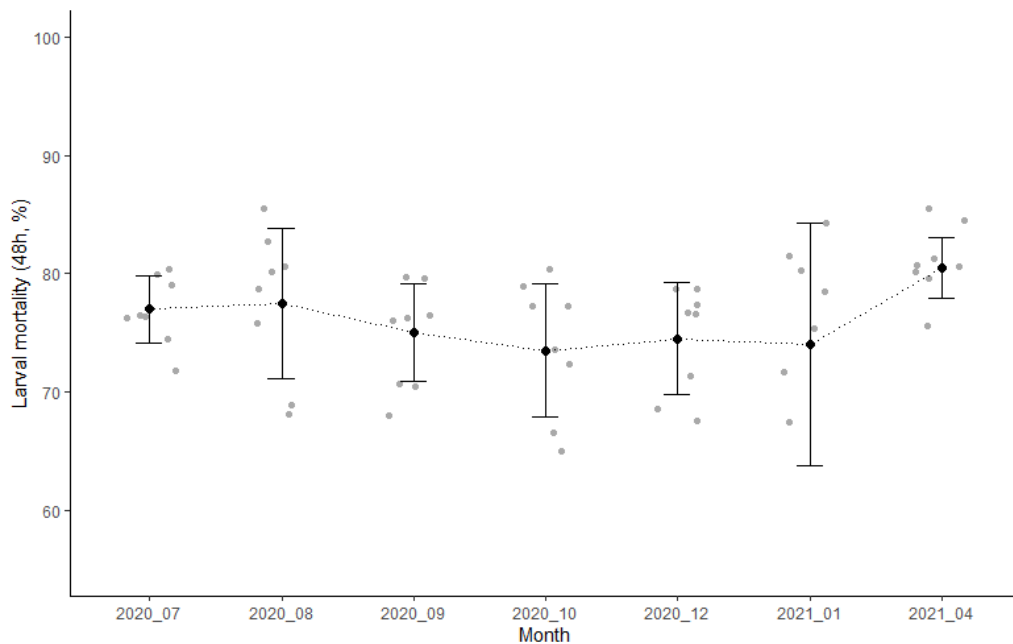


673

674 In the end, this study showed that although chemical changes, consisting of a decrease in  
675 rubrynolide content over time, were observed during controlled storage of the sawmill residues,  
676 these changes had no impact on the larvicidal potential of the extracts obtained at the tested  
677 dose. The last step was therefore to evaluate the stability of the extract itself, stored in an  
678 amber flask under ambient conditions in the laboratory. The 48-h larval mortality is shown in  
679 Figure 12, and full data are available in Table S9. Mortality ranged from  $73.5 \pm 6\%$  to  $80.5 \pm$   
680  $3\%$ , with a mean value of  $76 \pm 6\%$ . The Kruskal-Wallis test yielded a  $p$ -value of 0.08 (Chi-  
681 squared = 11.15, df = 6), highlighting the lack of a statistically significant difference in larval  
682 mortality as a function of extract storage time.

683

684 **Figure 12:** Percent mortality and standard error of the larval test exposing *Aedes aegypti* CAY  
685 F1 larvae to 30- $\mu$ g/ml *Sextonia rubra* extract during a 9-month extract storage period.



686

687

688 Altogether, these results are of particular interest when it comes to the technological and  
689 economical potential of *S. rubra* sawmill residues and extracts, as they demonstrate the good  
690 stability of these two resources over relatively long storage periods without adverse effects on  
691 their biological properties. However, these assays were performed under controlled conditions  
692 (storage of sawdust at low humidity and light, conservation of the extract in the laboratory at  
693 25°C), and care must be taken under industrial conditions, especially when it comes to the  
694 storage of sawmill residues (Cabalova et al., 2021; Grenier et al., 2022; Halmemies et al.,  
695 2022; Khan et al., 2014).

696

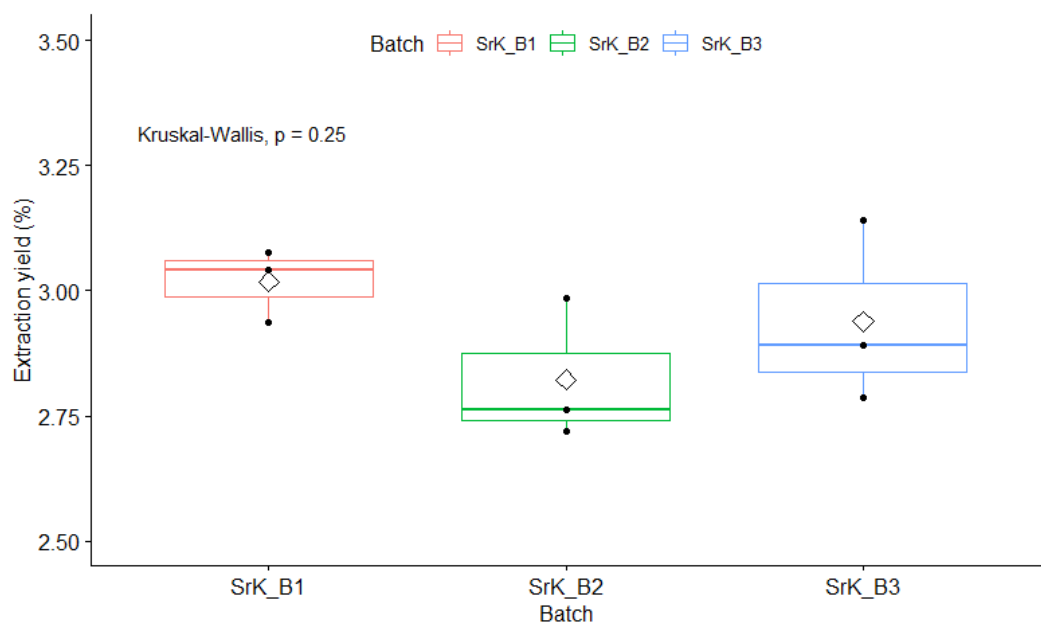
697 3.6. Valorization of *S. rubra* sawmill residues for the production of larvicidal  
698 extracts in French Guiana: a biorefinery perspective?

699 To complete this dataset, solvent extraction was performed for sawdust samples from the  
700 Kourou sawmill (SrK) ground to a standard particle size of 500  $\mu\text{m}$ . The mean extraction yield  
701 for SrK samples was  $2.9 \pm 0.1\%$ . The results obtained for the three SrK1, SrK2 and SrK3  
702 batches, collected as described in the experimental section, are shown in Figure 13. The full  
703 dataset is available in Table S10. These values are consistent with ethyl acetate extraction  
704 yields previously measured for *S. rubra* (Houël et al., 2017; Rodrigues et al., 2010).

705

706 **Figure 13:** Comparison of the extraction yields obtained for batches of *S. rubra* SrK sawdust  
707 (Kourou sawmill) from commercial forest waste (grain size  $\leq 500 \mu\text{m}$ ). The three batches  
708 (SrK\_B1, SrK\_B2 and SrK\_B3) correspond to three successive days of *S. rubra* sawing, and  
709 thus sawdust collection.

710



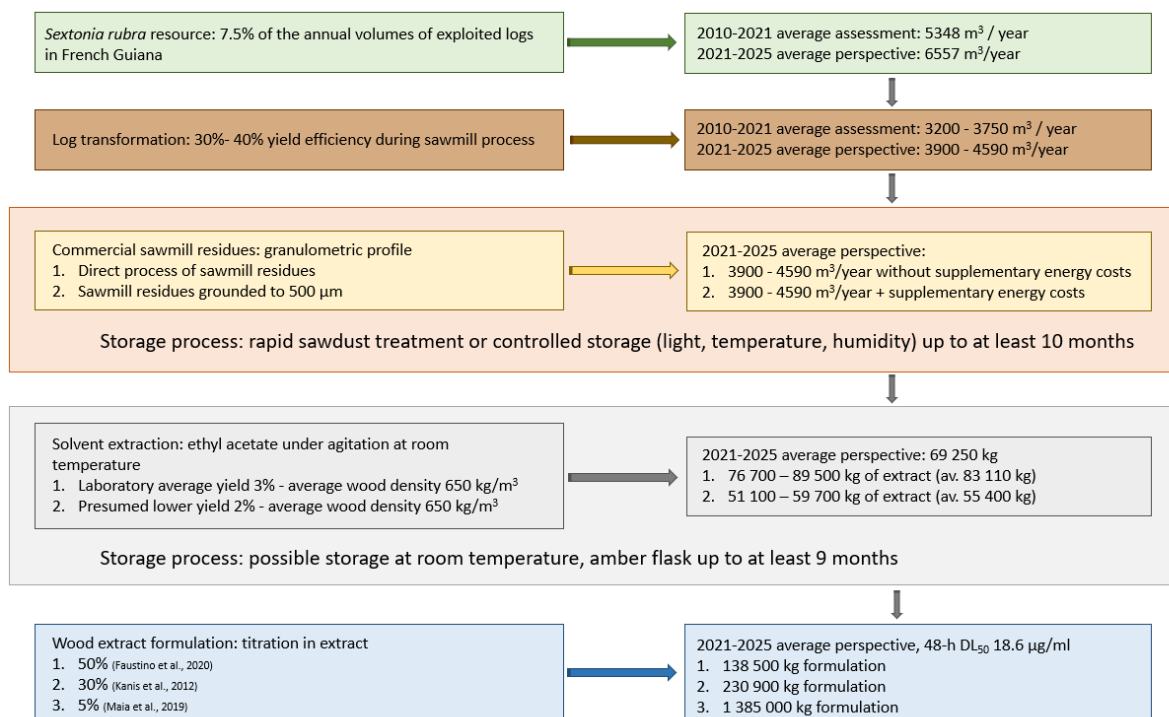
711

712

713 In order to progress towards TRL (technology readiness level) implementation, the different  
714 parameters highlighted in this study were finally summarized, and a preliminary technical route  
715 was illustrated (Figure 14). In particular, the French National Forest Office (ONF) production  
716 outlook for the next few years (2021-2025) foresees a 20% increase in log harvesting of *S.*  
717 *rubra* compared to the 2010-2021 period, due to the increase in wood needs for construction  
718 and energy correlated with the strong demographic growth observed in French Guiana (CRFB,  
719 2019). Consequently, the annual volume of available sawmill residues will also increase. As  
720 sawmill residues include at once bark, sapwood and sawdusts, the lower values presented in  
721 Figure 14 for lumber conversion should be the closest to the actual conditions (DAAF, 2016).  
722 Another positive aspect concerning a possible industrial valorization is that the recovery of  
723 sawmill by-products requires a certain concentration of supply to limit logistic costs. This could  
724 be achieved in French Guiana, as 5 sawmills account for 94% of the annual production of *S.*  
725 *rubra* logs. Regarding the processing of sawdust, the experimental design should be discussed  
726 with both sawmillers and extracts manufacturers, to define the necessary milling and  
727 processing steps and associated expenses (capital, equipment and investments, energy...),  
728 and evaluated by a full techno-economic analysis (García-Velásquez et al., 2023; Slegers et  
729 al., 2020; Wijeyekoon et al., 2021). In particular, the values given for the extraction yields are  
730 based on our laboratory protocol, but could be improved through the optimization of extraction  
731 procedures (Fernández-Agulló et al., 2015). The last step, regarding the preparation of a *S.*  
732 *rubra*-based formulations, is the subject of a second part of this project, and will be addressed  
733 in a future study.

734

735 **Figure 14:** Preliminary information for a biorefinery process: prospective valorization of *S.*  
736 *rubra* sawmill residues in French Guiana for the production of larvicidal extracts against *Ae.*  
737 *aegypti*.



738

739

#### 740 4. Conclusion

741 The aim of this study was to search for extracts of interest that could be valorized in a  
 742 biorefinery perspective, given the abundance of the raw material, the simplicity of the  
 743 transformation process, the biological efficacy; and the impact of the storage conditions. Our  
 744 findings demonstrated that commercial sawmill residues of *S. rubra* could be considered as a  
 745 good source of larvicidal extracts for biocontrol of *Ae. aegypti*. These extracts showed a  
 746 remarkable 48-h DL<sub>50</sub> value of 18.6 µg/mL against a pyrethroid-resistant wild strain, and were  
 747 obtained with ethyl acetate, an environment- friendly solvent than can be denoted as a green  
 748 solvent. Notably, the particle size profiles obtained independently of geographical origin  
 749 indicated that few pre-treatment steps would be necessary before applying the solvent  
 750 extraction process, thus allowing optimization of time and energy costs. Moreover, we pointed  
 751 out the importance of adequate storage to ensure that the chemical and biological properties  
 752 of the sawdust are maintained. By fulfilling these conditions, even a 10-month storage period  
 753 resulted in bioactive extracts. Furthermore, the were effective against *Ae. aegypti* larvae even

754 after 9 months of storage at room temperature. This stability duration pled in favor of the  
755 technical relevance of *S. rubra* for industrial valorization. Overall, the valorization of *S. rubra*  
756 sawmill residues could represent an important economic benefit in French Guiana, from the  
757 forestry sector to the natural products industry. This application in the field of the green  
758 chemistry would be complementary to the valorization of sawmill wastes in biomass power  
759 plants, as the latter only use the resources situated at the very end of the woody biomass  
760 supply chain.

761

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769 wood extracts as larvicidal products in the framework of the SextoniAedes project.

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