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Towards the optimization of botanical insecticides research: *Aedes aegypti* larvicidal natural products in French Guiana

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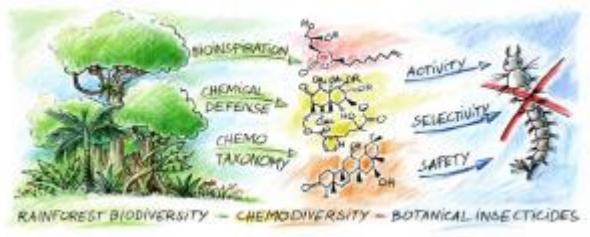
Abstract :

Natural products have proven to be an immeasurable source of bioactive compounds. The exceptional biodiversity encountered in Amazonia, alongside a rich entomofauna and frequent interactions with various herbivores is the crucible of a promising chemodiversity. This prompted us to search for novel botanical insecticides in French Guiana. As this French overseas department faces severe issues linked to insects, notably the strong incidence of vector-borne infectious diseases, we decided to focus our research on products able to control the mosquito *Aedes aegypti*. We tested 452 extracts obtained from 85 species originating from 36 botanical families and collected in contrasted environments against an *Aedes aegypti* laboratory strain susceptible to all insecticides, and a natural population resistant to both pyrethroid and organophosphate insecticides collected in Cayenne for the most active of them. 8 species (*Maytenus oblongata* Reissek, Celastraceae; *Costus erythrothyrsus* Loes., Costaceae; *Humiria balsamifera* Aubl., Humiriaceae; *Sextonia rubra* (Mez) van der Werff, Lauraceae; *Piper hispidum* Sw.,

Piperaceae; *Laetia procera* (Poepp.) Eichl., Salicaceae; *Matayba arborescens* (Aubl.) Radlk., Sapindaceae; and *Cupania scrobitulata* Rich., Sapindaceae) led to extracts exhibiting more than 50% larval mortality after 48h of exposition at 100 µg/mL against the natural population and were considered active. Selectivity and phytochemistry of these extracts were therefore investigated and discussed, and some active compounds highlighted. Multivariate analysis highlighted that solvents, plant tissues, plant family and location had a significant effect on mortality while light, available resources and vegetation type did not. Through this case study we highlighted that plant defensive chemistry mechanisms are crucial while searching for novel insecticidal products.

Graphical abstract

French Guiana biodiversity was explored for the search of novel larvicidal products against both insecticide-susceptible and -resistant *Aedes aegypti* populations.



Highlights

► French Guiana biodiversity was explored for the search of novel botanical insecticidal products ► 452 extracts from 85 plant species were tested for larvicidal activity against *Aedes aegypti* ► Bioassays included both insecticide-susceptible and -resistant *Ae. aegypti* populations *Sextonia rubra* wood extract and its main components were highlighted as promising products ► Correlations between extracts larvicidal activity and plants chemical defense were investigated

Keywords : Mosquito larvicides ; Culicidae ; Amazonian chemodiversity ; screening optimization ; quasi-Poisson generalized linear model ; chemical defense

1. Introduction

Although botanical insecticides are at the origin of all insecticidal compounds, they have been laid or put aside by the agrochemical industry. However due to the great damages caused by the overuse of synthetic compounds, natural products and molecules obtained from plants are again considered suitable pest control alternatives (George et al. 2014; Gerwick and Sparks 2014; Isman 2015). The considerable needs, efforts, challenges and limitations of this research are in particular described in a recent review, also presenting a wide range of plant extracts tested for larvicidal activity against various mosquitoes (Pavela et al., 2019).

Intensification of research on natural insecticides is in particular due to the crucial need of effective products to control mosquito vectors of pathogens, particularly viruses and *Plasmodium* parasites (Benelli 2015; Benelli and Mehlhorn 2016). Indeed during last years, the world has regularly experienced the emergence or re-emergence of arthropod-borne viruses such as yellow fever, dengue, chikungunya, and more recently Zika viruses. As

vector control remains an important, if not the sole tool to fight diseases spread, this increasing number of outbreaks alongside the expansion of insecticide resistance urge the development of novel molecules to control invasive mosquito populations such as *Aedes aegypti* (L., 1762) and *Aedes albopictus* (Skuse, 1895) (Carvalho and Moreira 2017; Fauci and Morens 2016; Faucon et al. 2015; Higgs and Vanlandingham 2015; Moyes et al. 2017). In particular *Ae. aegypti* Linnaeus (Diptera: Culicidae) is a cosmopolitan species originating from Africa but now well-established in all tropical and subtropical regions. This mostly diurnal anthropophilic mosquito is foud in urban communities and surrounding area, its presence being favoured by the existence of artificial breeding sites such as used tires, water tanks or flower pots. Its opportunistic behavior, high adaptation ability and biological characteristic such as eggs resistant to dessication, alongside with trade globalization and rapid urbanization are some keys of this species' ecological success (Carvalho and Moreira 2017; Simmons et al., 2012; Abilio et al., 2018). As sessile organisms, plants must have developed a wide range of secondary metabolites as defense compounds against predators and pests during their evolution (Agrawal and Weber 2015; Fraenkel 1959). The exceptional biodiversity of plants, entomofauna and herbivores in the tropics leads to a promising chemodiversity, due to the constant and dynamic interactions between plants and their environment (Becerra 2007; Ehrlich and Raven 1964; Richards et al.2015). Some factors were highlighted for playing a major role in the effectiveness of defense. According to the optimal defense theory, the allocation of defense chemicals is driven by the predation pressure exercised on a given plant organ, and the fitness value of this organ for the plant (McCall and Fordyce 2010; McKey 1974). Besides, not only this fitness value but also resources from a given environment would drive both the type and the amount of secondary metabolites (Coley et al. 1985; Endara and Coley, 2011). Open environments also represent places of higher herbivorous insects' abundance, and therefore larger insects-plants interactions, which could lead to the production of more, and/or more diverse insecticidal compounds (Lamarre et al. 2012). The type of defense may also differ between plants. Long-living, slow-growing species including woody plants would allocate resources to highly

concentrated quantitative defenses such as polyphenols and tannins, while short-lived species, e.g. herbaceous plants, would synthetize smaller amount of low molecular weight toxic compounds such as alkaloids, phenolic compounds or cyanogenic glycosides (Feeny 1976; Rhoades and Cates 1976; Smilanich et al. 2016).

For a few years, our team has therefore built a collection of plant extracts from ecologically contrasted Amazonian environments, including long-lasting trees and herbaceous plants, extracted from different plant organs. The objective was to consider plants having various growth-defense trade-offs in order to potentially improve our capacity to discover insecticidal compounds, and investigate ecological trends governing insecticidal properties. This approach has been inspired by the concept of “human chemical defenses” presented by Berenbaum (Berenbaum 1995). Literature-based chemotaxonomy was also included as a criterion for plant selection in our search for novel larvicidal extracts and compounds. As a consequence of the project, some methodological issues had to be discussed considering the huge amount of scientific literature already dealing with botanical insecticides research (Isman and Grieneisen 2014). The present contribution therefore also addresses some of these issues through illustrative examples encountered along the study, in an attempt to optimize plant screening for natural insecticides discovery.

2. Materials and methods

2.1. Plant material

All plant species (Table 1) were collected in French Guiana. They are not protected species and their collection was allowed without restriction at the concerned locations. Collection authorizations were given by the ONF (National Forest Office) where necessary. Herbarium vouchers were deposited in French Guiana Herbarium (CAY) where specialists confirmed botanical identification. All collection data are available at: http://publish.plantnet-project.org/project/caypub.

Botanical families	Plant species	CAY	Location [a]	Plant part
Annonaceae	*Anaxagorea dolichocarpa* Sprague & Sandwith	Odonne 721	Ko	Leaves, stems
	Guatteria ouregou (Aubl.) Dunal	Odonne 718	Ko	Leaves, stems
	Xylopia cayennensis Maas	Odonne 788	Ma	Leaves, bark
	Xylopia frutescens var. *ferruginea* R.E. Fr.	Odonne 774	Ma	Leaves, stems, bark
Apocynaceae	*Tabernaemontana siphilitica* (L.f.) Leeuwenb.	Odonne 724	Ko	Aerial parts
	Lacmellea aculeata (Ducke) Monach.	Odonne 749	Si	Leaves, stems
Asteraceae	*Bidens cynapiifolia* Kunth	Odonne 760	Mc	Whole plant
Bignoniaceae	*Handroanthus capitatus* (Bureau & K. Schum.) Mattos	Odonne 795	Rg	Leaves, stems
	Adenocalymma moringifolium (DC.) L.G.Lohmann	Odonne 727	Ko	Aerial parts
Boraginaceae	*Varronia schomburgkii* (DC.) Borhidi	Odonne 789	Ma	Aerial parts
Celastraceae	*Maytenus oblongata* Reissek	Odonne 726	Ko	Leaves, stems
	Maytenus sp.	Odonne 797	Rg	Leaves and fruits, stems
Chrysobalanaceae	*Couepia bracteosa* Benth.	Odonne 775	Ma	Leaves, stems
	Licania affinis Fritsch	Odonne 716	Ko	Leaves, stems
Clusiaceae	*Clusia palmicida* Rich.	Odonne 798	Rg	Leaves, stems
Combretaceae	*Terminalia amazonia* (J.F. Gmel.) Exell	Odonne 783	Ma	Leaves, bark
Convolvulaceae	*Ipomoea leprieurii* D.F. Austin	Odonne 791	Rg	Aerial parts
Costaceae	*Costus erythrothyrsus* Loes.	Odonne 742	Si	Leaves, stems, inflorescence
	Costus cf *spiralis* (Jacq.) Roscoe	Houël 3	Rm	Inflorescence
	Costus spiralis var. *villosus* Maas	Houël 4	Ko	Inflorescence
	Costus spiralis var. *villosus* Maas	Houël 5	Ro	Inflorescence
Cyperaceae	*Scleria cyperina* Willd. ex Kunth	Odonne 793	Rg	Aerial parts

Dilleniaceae	*Tetracera asperula* Miq.	Odonne 781	Ma	Leaves, fruits
Euphorbiaceae	*Cnidoscolus urens* (L.) Arthur	Odonne 790	Mc	Aerial parts
	Conceveiba guianensis Aubl.	Odonne 722	Ko	Leaves, stems
	Croton guianensis Aubl.	Odonne 786	Ma	Leaves
	Croton macradenis Görts & Punt	Eparvier 202	Mo	Aerial parts
	Croton matourensis Aubl.	Eparvier 167	Mt	Leaves, bark
	Croton nuntians Croizat	Eparvier 199	Si	Leaves, stems
	Croton nuntians Croizat	Odonne 743	Si	Leaves, stems
	Sapium argutum (Müll. Arg.) Huber	Odonne 794	Rg	Leaves, stems
Fabaceae	*Alexa wachenheimii* Benoist	Odonne 719	Ko	Leaves, bark
	Bocoa prouacensis Aubl.	238 [b]	Si	Bark
	Chamaecrista desvauxii var. *saxatilis* (Amshoff) H.S. Irwin & Barneby	Odonne 806	Rg	Aerial parts
	Chamaecrista diphylla (L.) Greene	Odonne 758	Mc	Aerial parts
	Dalbergia monetaria L.f.	Odonne 762	Mc	Leaves, stems
	Desmodium barbatum (L.) Benth.	Odonne 746	Si	Whole plant
	Dimorphandra polyandra Benoist	Odonne 779	Ma	Leaves, bark
	Entada polystachya (L.) DC.	Odonne 759	Mc	Leaves, stems
	Enterolobium schomburgkii (Benth.) Benth.	Forget 4976	Si	Wood, bark
	Inga alba (Sw.) Willd.	Moretti 1129	Si	Wood
	Inga virgultosa (Vahl) Desv.	Odonne 805	Rg	Leaves, stems
	Muellera frutescens (Aubl.) Standl.	Eparvier 108B	Mo	Leaves
	Macrolobium bifolium (Aubl.) Pers.	Odonne 725	Ko	Leaves, stems
	Macrolobium guianense (Aubl.) Pulle	Odonne 785	Ma	Leaves, wood

	Ormosia coutinhoi Ducke	Odonne 717	Ko	Leaves, stems
	Senna quinquangulata (Rich.) H.S. Irwin & Barneby	Odonne 738	Si	Leaves, stems
	Spirotropis longifolia (DC.) Baill.	Eparvier 137	Si	Wood, bark, roots
	Stylosanthes guianensis (Aubl.) Sw.	Odonne 792	Rg	Aerial parts
	Swartzia guianensis (Aubl.) Urb.	Odonne 715	Ko	Leaves, stems
	Vigna luteola (Jacq.) Benth.	Odonne 764	Mc	Aerial parts
Humiriaceae	*Humiria balsamifera* Aubl.	Eparvier 101	Mc	Wood
	Humiria balsamifera Aubl.	Odonne 784	Ma	Bark
Lauraceae	*Licaria cannella* (Meisn.) Kosterm.	Silland 16	Rg	Wood
	Sextonia rubra (Mez) van der Werff	1039 [b]	Si	Bark
	Sextonia rubra (Mez) van der Werff	Rodrigues 12	Rg	Wood
Loranthaceae	*Phthirusa* sp.	Odonne 720	Ko	Leaves, stems
Lythraceae	*Cuphea blackii* Lourteig	Odonne 796	Rg	Aerial parts
Malpighiaceae	*Byrsonima aerugo* Sagot	Odonne 780	Ma	Leaves
	Byrsonima crassifolia (L.) Kunth	Odonne 755	Mc	Leaves, bark
	Byrsonima spicata (Cav.) DC.	Odonne 754	Mc	Leaves, wood, bark
Malvaceae	*Eriotheca surinamensis* (Uittien) A. Robyns	Odonne 801	Rg	Leaves
	Sterculia pruriens (Aubl.) K. Schum	1058 [b]	Si	Bark
Melastomataceae	*Ernestia granvillei* Wurdack	Odonne 804	Rg	Aerial parts
Meliaceae	*Azadirachta indica* A. Juss	Odonne 712	Ko	Leaves
	Guarea guidonia (L.) Sleumer	Odonne 756	Mc	Leaves, stems
Moraceae	*Bagassa guianensis* Aubl.	n.i. [c]	Si	Bark
Myrtaceae	*Myrcia saxatilis* (Amshoff) McVaugh	Odonne 799	Rg	Leaves, stems
Orobanchaceae	*Anisantherina hispidula* (Mart.) Pennell	Odonne 757	Mc	Whole plant

Piperaceae	*Piper hispidum* Sw.	Odonne 741	Si	Leaves, stems
Polygalaceae	*Polygala longicaulis* Kunth	Odonne 787	Ma	Whole plant
Rubiaceae	*Posoqueria longiflora* Aubl.	Odonne 723	Ko	Leaves
	Tocoyena guianensis K. Schum.	Odonne 802	Rg	Aerial parts
	Sipanea pratensis Aubl.	Odonne 803	Rg	Aerial parts
Salicaceae	*Banara guianensis* Aubl.	Odonne 748	Si	Leaves, stems
	Casearia grandiflora Cambess.	Odonne 777	Ma	Leaves, wood, bark
	Laetia procera (Poepp.) Eichl.	1003 [b]	Si	Bark
	Laetia procera (Poepp.) Eichl.	424 [b]	Si	Bark
	Laetia procera (Poepp.) Eichl.	Odonne 771	Mc	Bark
Sapindaceae	*Cupania scrobiculata* Rich.	Odonne 778	Ma	Leaves, stems, fruits
	Matayba arborescens (Aubl.) Radlk.	Odonne 776	Ma	Leaves, stems, fruits
	Paullinia sp.	Odonne 713	Si	Leaves, stems
	Paullinia pinnata L.	Odonne 763	Mc	Aerial parts
Sapotaceae	*Manilkara huberi* (Ducke) A. Chevalier	Riéra 1904	Si	Wood, bark
Simaroubaceae	*Quassia amara* L..	Odonne 714	Rm	Stems
Siparunaceae	*Siparuna guianensis* Aubl.	Odonne 747	Si	Leaves, stems
Solanaceae	*Cestrum latifolium* Lam.	Odonne 761	Mc	Leaves, stems
	Solanum leucocarpon Dunal	Odonne 740	Si	Leaves, stems
	Solanum stramoniifolium Jacq.	Odonne 751	Si	Aerial parts
	Solanum subinerme Jacq.	Odonne 752	Si	Aerial parts
Vochysiaceae	*Erisma uncinatum* Warm.	514 [b]	Si	Bark

[a] Legend: Régina (Rg), Roura (Ro), Matoury (Mt), Rémire-Montjoly (Rm), Montsinéry-Tonnegrande (Mo), Macouria (Mc), Kourou (Ko), Sinnamary (Si), Mana (Ma)

[b] Trees from a permanent plot (St Elie) in Sinnamary. This permanent research plot hosts up to 800 identified trees. The systematic identification of the trees was performed at the IRD herbarium in Cayenne where a voucher sample is deposited

[c] Not integrated in Cayenne herbarium. Bagassa guianensis was collected in the framework of other research projects and botanical identification was made in situ by professional forest workers

Table 1 Botanical families, plant species, voucher number (Cayenne herbarium - CAY), location and plant parts collected for testing against 3rd-4th instar larvae of *Ae. aegypti* L. (Diptera: Culicidae)

Plants were collected along an E/W geographical gradient (Figure 1) in the following locations: Régina (Rg), Roura (Ro), Matoury (Mt), Rémire-Montjoly (Rm), Montsinéry-Tonnegrande (Mo), Macouria (Mc), Kourou (Ko), Sinnamary (Si), Mana (Mn). The various environment types were the following: *terra firme* forest, forest edges, white-sand forest, river bank, dry savannah, coastline, inselberg and ruderal/disturbed areas. To perform multivariate analysis, these environments were described according to the available light (few light: forest, to strong light: open environment such as savannah or inselberg) and resources (from poor environment such as inselbergs to abundant resources environment such as forest or river bank) at the collection place. The type of vegetation (life-forms) was also characterized (temporary vegetation, secondary / low or slightly ligneous vegetation, ligneous species, large trees). Collected plant organs were: bark, wood, stems, roots, leaves, aerial parts, whole plant, inflorescence and fruits depending on the plant.

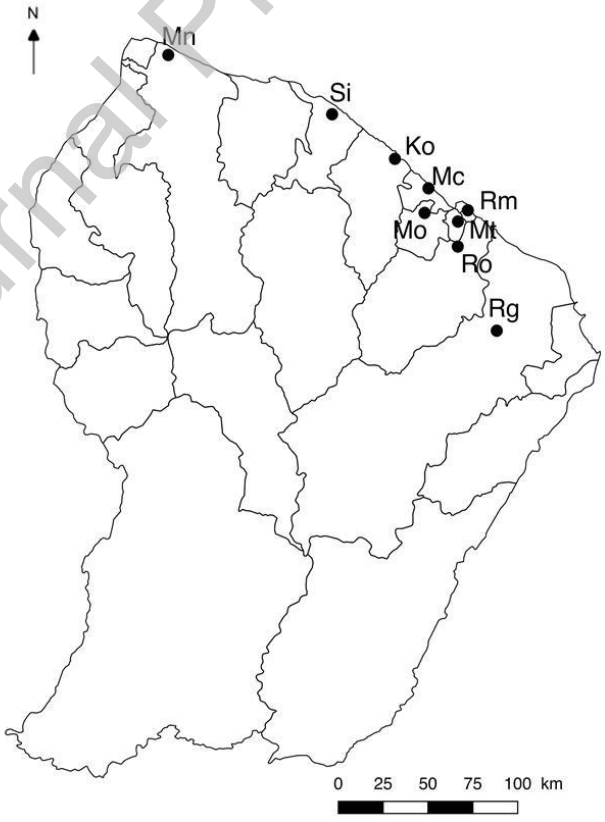


Fig. 1 Repartition of the collection localities. Legend: Régina (Rg), Roura (Ro), Matoury (Mt), Rémire-Montjoly (Rm), Montsinéry-Tonnegrande (Mo), Macouria (Mc), Kourou (Ko), Sinnamary (Si), Mana (Ma)

2.2. Extraction

All plant parts were air-dried (room temperature, 10% air relative humidity) and finely ground into powder prior to extraction. Plant powders (30 g) were successively extracted at room temperature by maceration during 24h under stirring, using either ethyl acetate (3 x 150 ml) followed by methanol (1 x 150 ml), or petroleum ether (3 x 150 ml) followed by boiling water (1 x 150 ml). After each extraction, the solution was filtered and the solvent removed by evaporation under reduced pressure with a SpeedVac™ concentrator (Savant SPD121P, Thermo Scientific). The resulting crude extracts (up to 4 extracts for each plant part of each species) were stored in a freezer at -18°C until assayed.

2.3. Evaluation of larvicidal activity

Insect collection and rearing, cup assay and data analysis were performed as previously described (Touré et al. 2017). Two *Aedes aegypti* (Diptera: Culicidae) strains were used for testing the extracts and compounds. The laboratory strain Paea, collected in French Polynesia, and maintained for a decade in the insectary at the Institut Pasteur de la Guyane, French Guiana, is susceptible to all insecticides. The Cayenne natural population is resistant to both pyrethroid and organophosphate insecticides and is a first generation (F1) strain reared from wild-caught larvae (F0) (Dusfour et al. 2011). The choice to perform a two-step screening was based on the recommendations made by Cos et al. for antimicrobial screening to develop a stronger proof of concept (Cos et al. 2006). Indeed LC_{50} could increase 100 times in *Ae. aegypti* resistant populations compared to susceptible ones (Lima et al. 2011). Late third or early fourth-instar larvae were used in the tests. All extracts were

investigated using the WHO procedure for testing of mosquito larvicides (WHO 2005). For each bioassay, 25 larvae of each strain were transferred to cups containing 99 mL of distilled water and 1 mL of the tested product diluted in ethanol, at the suitable concentration, and four cups, representing a total of 100 larvae, were used for each tested concentration. For the determination of mortality rates, the final concentration was 100 µg/mL and for LC_{50} calculation, concentrations leading from 0 to 100% mortality were tested. Larval mortality was recorded 24 and 48 h after exposure. Control treatments were performed for each test with 1 mL of ethanol, and deltamethrin (0.05 µg/mL) was used as a positive control in the case of the laboratory strain Paea. *Muellera frutescens* (Aubl.) Standl. (Fabaceae), of which leaves were previously described to contain the rotenoid compounds rotenone, tephrosin and deguelin, and to be toxic against *Ae. aegypti* mosquito larvae, was included in the screening to serve as a botanical positive control in order to validate the test protocol (Falkowski et al. 2016; Nirma et al. 2009). Abbott's formula was applied to mortalities if mortality in the control was between 5% and 20% (Abbott 1925). The test was invalidated if mortality in the control was greater than 20%. Lethal doses were obtained by a probit regression under a general linearized model [BioRssay 6.1. script in R environment version 3.2.0 (https://www.r-project.org/)].

2.4. Cytotoxicity assays

Cytotoxicity assays were conducted with KB (nasopharyngeal epidermoid carcinoma) and MRC5 (normal lung tissue of a 14-week-old male foetus) cell lines using the procedure described by Tempête et al. (Tempête et al. 1995). Docetaxel was used as positive control.

2.5. Ecotoxicological assessment on non-target species, Daphnia magna *and* Chironomus riparius

Ecotoxicity assays were adapted from the guidelines of the “Immediate Immobilization Test” (OECD No. 202) for *Daphnia magna* (Straus, 1820) and the “Immediate Immobilization Test” (OECD No. 235) for *Chironomus riparius* (Meigen, 1804). The extracts were tested only at the LC_{50} value defined from the *Ae. aegypti* Paea strain sensitivity for each extract. Three conditions were tested: control, control/solvent, and LC_{50}, with four replicates per condition. The physicochemical measurements (pH, dissolved oxygen, conductivity) were performed with measuring devices (sensors). The remaining measures (chlorine, nitrites, nitrates, phosphates) were performed with aquarium strips. Photoperiod and temperature were recorded using a “templight” recorder throughout the test period, from clutch incubation until the end of the exposure.

2.6. Phytochemical studies

2.6.1. General remarks

^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra at 100.6 MHz on a Varian 400 MR spectrometer equipped with a 5 mm inverse probe (Auto X PGF 1H/15N-13C). Samples were dissolved in deuterated chloroform ($CDCl_3$) in 5 mm tubes as stated. Chemical shifts are in ppm downfield from tetramethylsilane (TMS), and coupling constants (*J*) are in Hz (s stands for singlet, d for doublet, t for triplet, q for quartet, m for merduplet, br for broad). TLC analyses were performed using ALUGRAM®SIL G/UV_{254} plates, eluted with petroleum ether 90:10 and revealed using a solution of 1% $KMnO_4$ in water.

Water (HPLC grade) was obtained from a Milli-Q system (Milli-Q plus, Millipore Bedford, MA). HPLC analyses were performed on a Discovery C18 column (15 cm x 4.6 mm, 5 µm, Supelco) at 1 mL/min using a Waters HPLC system equipped with a W2996 photodiode array absorbance detector and a W2424 light-scattering detector. HPLC semi-preparative chromatography was performed at 15 mL/min on a Discovery C18 column (15 cm x 21.2 mm, 5 µm, Supelco) using a Waters HPLC system equipped with a W600 pump and a W2487 double wavelength UV detector (Waters).

2.6.2. Costus erythrothyrsus *Loes. (Costaceae) phytochemical study*

C. erythrothyrsus inflorescence ethyl acetate extract was purified by column chromatography using a petroleum ether / ethyl acetate gradient from 100:0 to 10:90 and eventually 100% methanol. Ten fractions were gathered according to their TLC profile. Fraction F4 eluted with petroleum ether / ethyl acetate 85:15 exhibited 72% larvicidal mortality against *Ae. aegypti* Paea laboratory strain at 100 µg/mL and 100% larvicidal mortality against Cayenne resistant strain at the same concentration, and its chemical composition was therefore investigated using NMR. TLC profiles from the crude extract and fraction F4 were also compared to standard lipids L13-0521 (VHOSO, Very High Oleic Sunflower Oil fatty acids), L13-0001 (Linseed oil fatty acids including 50% linolenic acid, 21% linoleic acid and 13% oleic acid), E12-1986 (stearic acid), L14-0146 (hydrogenated VHOSO methylic ester – stearic acid) and P14-002 (VHOSO methylic ester – oleic acid) kindly provided by ITERG (Institut des Corps Gras, Pessac, France).

2.6.3. Maytenus oblongata *Reissek (Celastraceae) phytochemical study*

The thoroughgoing bioguided fractionation as well as isolation and identification of *M. oblongata* extract components were described in Touré et al. (2017).

2.6.4. Sextonia rubra *(Mez.) van der Werff (Lauraceae) phytochemical study*

Isolation of rubrenolide and rubrynolide was performed using HPLC semi-preparative chromatography according to previously described procedures (Fu et *al.* 2019) and their identification confirmed by NMR.

2.7. Multivariate analysis

The complete set of data used for multivariate analysis is available in Supporting Information (table S1). Multivariate analysis was conducted in R 3.2.0 environment. Z-scores were obtained from mortality data. This transformation gives the dataset a mean of 0 and a standard deviation of 1. A generalized linear model (GLM) using the quasi-Poisson distribution, logistic link function and a mixture of forwards and backwards selection was used to relate mortality responses to the technical, chemotaxonomic and environmental predictor variables. Pairwise comparisons were further performed with TukeyHSD test between modalities of each factor that were identified to have an effect on mortalities. Family, solvent, organ, light, resource, type of vegetation and location were thus selected as explanatory variables in our analyses.

3. Results and discussion

3.1. Larvicidal screening on susceptible and resistant Ae. aegypti strains

A total of 144 plant parts issued from 85 species belonging to 36 botanical families were collected during the project (Table 1). Fabaceae (24%) were the most represented, with 17 genera and 20 species. Euphorbiaceae, Annonaceae, Sapindaceae and Solanaceae represented from 5 to 8% of the collected species (Figure 2). The genera *Byrsonima*, *Croton* and *Solanum* were the most represented, with 3 to 4 species each. For two species (*Croton nuntians* Croizat, Euphorbiaceae, *Laetia procera* (Poepp.) Eichler, Salicaceae) 2 to 3 different samples of the same plant part were collected at different times and locations. It should be noticed that the Fabaceae family is one of the most cited in the literature for its insecticidal activity, being notably a source of rotenoids and in particular rotenone, a well-known, yet controversial botanical insecticide (Boulogne et al. 2012; Isman 2006; Pavela et al., 2019).

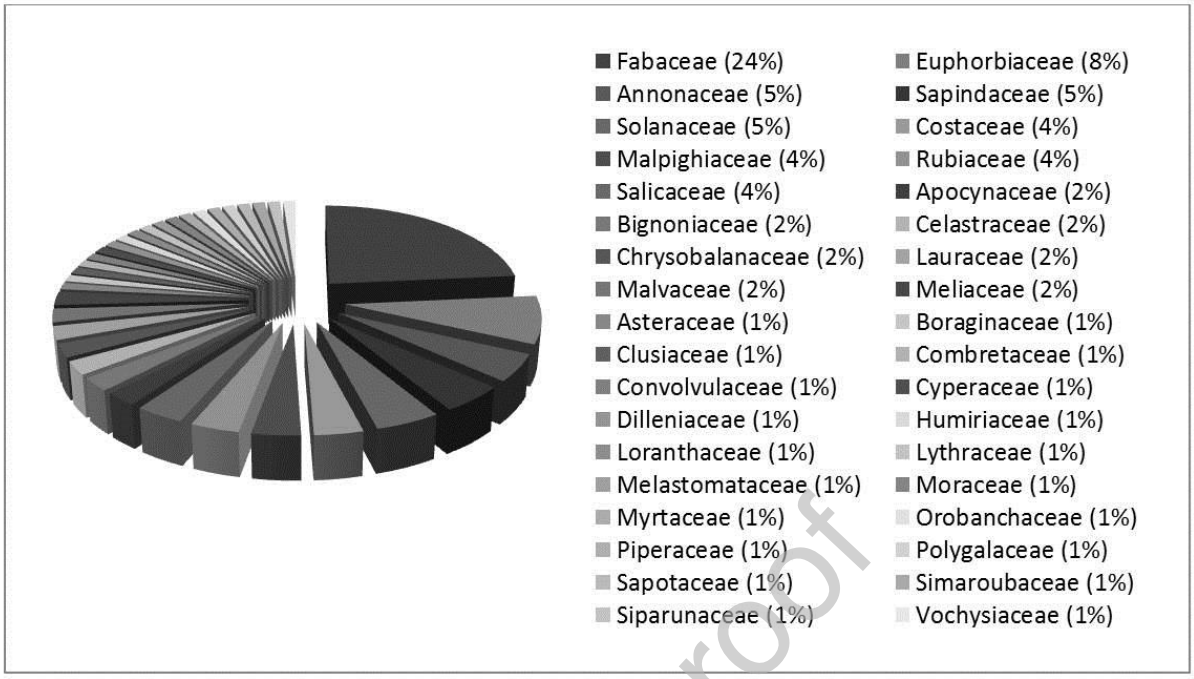


Fig. 2 Diversity of the collected species: the relative importance of the botanical families is shown in the pie chart (families are represented clockwise in the pie chart)

Eventually, 452 extracts were obtained and tested on *Ae. aegypti* Paea strain. The complete dataset is available in Supporting Information (Table S1). The extracts exhibiting more than 50% mortality after 48 h of exposition at 100 µg/mL were considered active, which is consistent with the requirements proposed by Pavela (2015). Fifteen botanical species thus led to 22 larvicidal extracts listed in Table 2. The active extracts on the Paea strain were then tested on a natural population of resistant Cayenne *Ae. aegypti* in order to obtain more selective and realistic results, thus improving the probability to highlight promising plant extracts for the search of new botanical insecticides.

The extracts exhibiting larvicidal mortality ≥ 50% after 48 h of exposition at 100 µg/mL against this resistant strain are highlighted in Table 2. Eventually, 8 species led to 11 larvicidal extracts against the Cayenne strain. Among the botanical families hosting these active species Celastraceae (Alvarenga and Ferro 2005; Deepa and Narmatha Bai 2010),

Lauraceae (Cuca-Suarez et al. 2012; Dias and Moraes, 2014), Piperaceae (Dorla et al. 2017; Lija-Escaline and al. 2015; Marques and Kaplan 2015), and Sapindaceae (Diaz and Rossini 2012), are particularly well described for their numerous insecticidal effects. These 8 species represents 9% of the collected species and 2% of the extracts. By comparison, among 94 extracts from 10 Brazilian plant species selected randomly or according to chemotaxonomic criteria,19 were considered to be effective against *Ae. aegypti* larvae, exhibiting LC_{50} < 250 µg/mL, including 6 (6.4%) extracts with LC_{50} < 100 µg/mL (Oliveira et al. 2010). Another 27 species identified from a screening performed on 83 Asteraceae belonging to 48 genera, promoted statistically significant mortality of *Ae. fluviatilis* (Lutz, 1904) 4th instar larvae, with 8 (9.6%) species leading to 50% or more mortality at 100 µg/mL (Macêdo et al. 1997). In terms of active extracts, these results are consistent with those observed in our screening, even if contrary to the example of the Asteraceae family, the selected plants in our case did not all belong to botanical families well-known for the insecticidal activities of their species. This could be an indication that selecting species on different criteria, e.g. the ecosystem, could also lead to interesting results.

Botanical families	Plant species [a]	Plant part	Solvent [c]	Extraction yield (%)	Mortality (%, Paea strain) ± SD	LC_{50} (µg/ml), Paea strain [d]	Mortality (%, Cayenne strain) ± SD [e]	LC_{50} (µg/ml), Cayenne strain [d]
Annonaceae	*Xylopia frutescens var. ferruginea*	Leaves	PE	3.0	54 ± 6.6		4 ± 0.0	
Asteraceae	*Bidens cynapiifolia*	Whole plant	EA	1.4	97 ± 1.0		5 ± 3.0	
Celastracee	***Maytenus oblongata***	**Stems**	**EA**	**1.5**	**98 ± 1.1**	**74.4 ± 2.5 [f]**	**91 ± 3.0**	**n.t.**
Chrysobalanaceae	*Licania affinis*	Leaves	PE	0.8	97 ± 1.1		2 ± 1.1	
	Licania affinis	Stems	PE	0.1	100 ± 0.0		n.t.	
Costaceae	***Costus erythrothyrsus (Odonne 742)***	**Inflorescence**	**EA**	**4.8**	**100 ± 0.0**	**45.0 (95%CI: 36.6-54.0)**	**100 ± 0.0**	**55.7 (95%CI: 49.8-61.2)**
	Costus erythrothyrsus (Odonne 742)	**Inflorescence**	**PE**	**3.9**	**97 ± 1.0**	**n.t.**	**99 ± 1.0**	**n.t.**
Euphorbiaceae	*Croton macradenis*	Aerial parts	PE	0.4	54 ± 3.8		5 ± 1.9	
Fabaceae	*Alexa wachenheimii*	Bark	PE	0.2	56 ± 2.8		2 ± 1.1	
	Muellera frutescens [b]	Leaves	PE	3.7	100 ± 0.0		n.t.	
	Muellera frutescens	Leaves	EA	4.3	100 ± 0.0		n.t.	
	Muellera frutescens	Leaves	M	4.6	97 ± 1.9		n.t.	
Humiriaceae	***Humiria balsamifera (Odonne 784)***	**Bark**	**EA**	**17.9**	**84 ± 4.3**	**49.0 (95%CI 35.8-64.8)**	**91 ± 1.9**	**45.0 (CI95% 39.0-51.2)**
Lauraceae	***Sextonia rubra (Rodrigues 12)***	**Wood**	**EA**	**4.2**	**100.0 ± 0.0**	**3.1 7 (95%CI 2.7-3.7)**	**100.0 ± 0.0**	**n.t.**
	Sextonia rubra (1039)	**Bark**	**EA**	**2.4**	**100.0 ± 0.0**	**n.t.**	**85.0 ± 6.0**	**n.t.**
Piperaceae	***Piper hispidum***	**Leaves**	**EA**	**7.0**	**62 ± 5.3**	**54.7 (95%CI 46.0-64.0)**	**84 ± 1.6**	**n.t.**
Salicaceae	***Laetia procera (1003)***	**Bark**	**PE**	**3.4**	**94 ± 2.0**	**33.5 (95%CI 28.0-39.8)**	**87 ± 3.4**	**61.0 (95%CI 49.8-77.7)**
	Laetia procera (1003)	**Bark**	**EA**	**2.7**	**63 ± 6.0**	**43.7 (95%CI: 33.9-57.3)**	**57 ± 7.2**	**65.9 (95%CI: 51.7-90.5)**
Sapindaceae	***Matayba arborescens***	**Fruits**	**EA**	**11.2**	**60 ± 2.8**	**76.9 (95%CI 67.7-86.7)**	**98 ± 1.1**	**40.5 (95%CI 34.1-46.2)**
	Matayba arborescens	**Fruits**	**PE**	**17.4**	**51 ± 6.6**	**n.t.**	**50 ± 6.8**	**n.t.**
	Cupania scrobiculata	**Fruits**	**EA**	**2.9**	**64 ± 1.6**	**105.3 (95%CI 86.6-136.5)**	**74 ± 3.8**	**102.5 (95%CI 80.4-145.4)**
Solanaceae	*Cestrum latifolium*	Stems	EA	0.7	58 ± 10.5		7 ± 1.9	

[a] Voucher number at Cayenne Herbarium (CAY) or tree number from a permanent plot in Sinnamary is indicated when several samples were previously collected

[b] *Muellera frutescens* was used as a botanical insecticide positive control in order to validate the test protocol

[c] PE: petroleum ether; EA: ethyl acetate; M: methanol

[d] LC_{50} values were calculated after 48h of exposition unless otherwise specified

[e] n.t.: not tested.

[f] LC_{50} value was calculated after 24h of exposition using the protocol descred in Touré et al. (2017)

Table 2 Active extracts (mortality ≥ 50% after 48 h of exposition at 100 µg/mL) against *Ae. aegypti* Paea and Cayenne strains 3rd-4th instar larvae. Extracts exhibiting larvicidal activities ≥ 50% against Cayenne resistant strain are highlighted in bold characters.

3.2. Selectivity of the active extracts and phytochemical discussion

In the global perspective of improving our knowledge about the selectivity of the extract in terms of bioactivity, cytotoxicity of the extracts highlighted as active on the *Ae. aegypti* Cayenne strain was then evaluated on two human cellular strains (KB cancerous cell line, MRC5 healthy cell line). Concurrently 3 randomly selected extracts (*Maytenus oblongata* Reissek, Celastraceae, *Matayba arborescens* (Aubl.) Radlk., Sapindaceae, and *Humiria balsamifera* Aubl., Humiriaceae) were tested for possible ecotoxicity against non-target species *C. riparius*, an aquatic diptera, and *D. magna*, a small planktonic crustacean. If the obtained values (inhibition of cellular growth for cytotoxic assay, and mortality for ecotoxicity) were too high, the extract was abandoned. These bioassays were used at this step of the screening to prevent further study of active non-selective extracts. Indeed, as stated by Isman and Grieneisen, studying the effect of botanical insecticides on human health is quite rare in the existing literature, as most botanicals are renowned for their low acute toxicity (Isman and Grieneisen 2014). However, plants can be highly toxic too and this parameter should clearly be taken into account in the context of the search for new insecticides of plant origin. The cytotoxicity results are presented in Table 3. *Cupania scrobitulata* Rich. (Sapindaceae) fruits extract was cytotoxic and was dropped. *M. arborescens* fruits extract was only moderately cytotoxic but was strongly ecotoxic with almost 100% of mortality against both *C. riparius* and *D. magna* at 100 µg/mL and was therefore dropped as well.

H. balsamifera bark ethyl acetate extract exhibited significant cyto- and ecotoxicity, with respectively 52±2% and 40±5% of growth inhibition against KB and MRC5 cells at 10 µg/ml, and leading to almost 100% of mortality against both *C. riparius* and *D. magna* at 80 µg/mL. The latter value is close to the LC_{50} values of 63.6 (CI95% 52.7-77.5) and 49.0 (CI95% 35.8-64.8) µg/mL measured at 24 and 48 h against the laboratory strain Paea. *H. balsamifera* is a large tree common in Amazonia and North-East Brazil. Numerous compounds were isolated from this species, including *trans*-isolongifolenone (Da Silva et al. 2004).[48] Interestingly, a

repulsive effect of this compound was described on *Ae. aegypti* and *Anopheles stephensi*, but also on other insects (Wang et al. 2013; Zhang et al. 2009). Moreover, t*rans*-isolongifolenone is described as odorless, whereas some of its derivatives have a characteristic woody smell (Zhang et al. 2009). It has to be noted that the sampled bark was strongly odoriferous, and we noticed in the field that this phenomenon was linked to a damage caused to the bark, leading to an abundant production of resinous product. It should therefore be checked if the more frequently encountered non-odoriferous barks also lead to larvicidal extracts, and if odoriferous isolongifolenone derivatives exhibit larvicidal activity. In the case of this extract, toxicity could be linked with the plant's response to stress due to mechanical damage, leading to the production of defensive compounds. If these molecules led to the discovery of a larvicidal extract, our results also highlight the fact that cyto- and ecotoxicity bioassays are essential in the evaluation of a potential new insecticidal product, as *H. balsamifera* bark extract showed to be non-selective against the various targets tested in our study. It should also be mentioned that *H. balsamifera* wood extract did not exhibit any larvicidal activity in our hands. It would be interesting to investigate if this difference is linked to the collected specimen or to a systematic difference in terms of chemical defenses allocation between the two tissues.

Botanical families	Plant species [a]	Plant part / Solvent [b]	Growth inhibition of KB cells, % ± SD [c]		Growth inhibition of MRC5 cells, % ± SD [c]	
			10 µg/ml	1 µg/ml	10 µg/ml	1 µg/ml
Celastracee	*Maytenus oblongata*	Stems (EA)	9±1	n.t.	8±6	n.t.
Costaceae	*Costus erythrothyrsus* (Odonne 742)	Inflorescence (EA)	0±1	0±10	0±1	0±1
Humiriaceae	*Humiria balsamifera* (Odonne 784)	Bark (EA)	52±2	5±2	40±5	18±4
Piperaceae	*Piper hispidum*	Leaves (EA)	22±5	0±1	0±4	0±1
Salicaceae	*Laetia procera* (1003)	Bark (PE)	18±2	0±1	45±5	4±1
Sapindaceae	*Matayba arborescens*	Fruits (EA)	24±6	n.t.	16±2	n.t.
	Cupania scrobiculata	Fruits (EA)	71±1	1±6	67±2	27±2

[a] Voucher number at Cayenne Herbarium (CAY) or tree number from a permanent plot in Sinnamary is indicated when several samples were previously collected

[b] PE: petroleum ether; EA: ethyl acetate; M: methanol

[c] PE: petroleum ether; EA: ethyl acetate; M: methanol

Table 3 Growth inhibition of KB (nasopharyngeal epidermoid carcinoma) and MRC5 (normal lung tissue of a 14-week-old male fetus) cell lines. Positive control: docetaxel induced 0.0005% survival at 1 µg/mL.

Species from the genus *Costus* are rhizomatous perennial herbs from the Costaceae family (Specht and Stevenson 2006). In our study, *C. erythrothyrsus* Loes. inflorescence ethyl acetate extract exhibited no cytotoxicity against human cell lines, which could make this extract a valuable candidate in the search of new botanical insecticides. The LC_{50} at 24 and 48 h were 69.1 (95% CI: 59.4-82.9) and 45.0 (95% CI: 36.6-54.0) µg/mL, respectively, against the Paea strain. Although the insecticidal activity of several *Costus* species has been described before in the literature, neither *C. eryhthothyrsus* nor *Costus* inflorescences were described for insecticidal activity (Pipithsangchan and Morallo-Rejesus 2005; Surendra Kumar et al. 2014). However, although the first extract exhibited strong larvicidal activity, no other positive result could be observed while testing inflorescences extracts from the same *C. erythrothyrsus* specimen at a later collection date, or inflorescences collected from other *Costus* species. Interestingly, we noticed that the first collected inflorescence had been damaged by some predators. Defense compounds may have been produced by the plant on this occasion, and further studies to investigate these mechanisms and the compounds responsible for the biological activity would thus be of great interest. In the case of this extract, bioguided fractionation allowed us to isolate an active mixture of 3 fatty acids. ^{1}H NMR spectrum was consistent with a mixture linolenic, linoleic and oleic acids (Figure S1) (Sacchi et al. 1997). This type of compounds were already found in a chemical analysis performed on the inflorescence of *Etlingera elatior* Jack, a plant from the Zingiberaceae family, which is in close relationship with Costaceae, and are known for their larvicidal activity (Harada et al. 2000; Jeevani Osadee Wijekoon et al. 2011; Rahuman et al. 2008; Ramsewak et al. 2001; Santos et al. 2017). Moreover, they were also highlighted for their role in chemical defense mechanisms, and more particularly induced defense against pathogenous organisms (Domingues et al. 2007; Rojas et al. 2014; Ryu et al. 2005). Therefore the fact that a single extract was found active might be correlated with the activation of defense mechanisms in response to herbivory damage and this observation could be the subject of complementary studies.

The case of *L. procera* petroleum ether bark extract also raised interesting questions concerning the activation of chemical defense mechanisms and the interest of studying several samples of a same species. *L. procera* is a long-lived pioneer tree, i.e., a fast-growing light-demanding species, characterized as an early colonizer of the Amazonian forest (Santos et al. 2012). Petroleum ether bark extract of *L. procera* N°1003 collected in the Saint Elie permanent investigation plot in Sinnamary (Si) led to a LC_{50} value of 33.5 (CI95% 28.0-39.8) µg/mL at 48 h against the laboratory strain Paea. However, no larvicidal activity was discovered while testing two other bark extracts. One inactive tree bark had been collected in the same mature forest area as the active bark (tree N°424), and the second one in a secondary forest close to dry savannahs in Macouria (Mc). Therefore, the activity described for the first sample could be due again to an increased production of toxic compounds by a single individual rather than an environmental effect of resources availability. Indeed, Jullian et al. already described the fact that bark extracts from the same tree N°1003, collected a few years before, led to the isolation of laetiaprocerine A and laetianolide A as major compounds, whereas casearlucine A and caseamembrol A were the main components of tree N°424 bark extract, alongside with small amounts of laetiaprocerine A and laetianolide A (Jullian et al. 2005). Preliminary phytochemical studies were performed but did not lead to a clear conclusion concerning the pure compounds responsible for the bioactivity of the extract. It would thus be interesting to pursue the evaluation of the larvicidal activity of the pure compounds, and correlate *L. procera* bark extracts chemical profiles and larvicidal activity for example through a metabolomic approach.

M. oblongata extracts were not cytotoxic against KB and MCR5 human cell lines at 10 µg/mL, and exhibited noticeable toxicity against *C. riparius* whereas it did not have any activity against *D. magna* at 75 µg/mL, as presented in a previously published article (Touré et al. 2017). This article also reported the isolation in *M. oblongata* extract of two new sesquiterpene alkaloids with a β-dihydroagrofuran skeleton and exhibiting significant activity against Paea strain *Ae. aegypti* larvae. Whereas published elsewhere, this work was

performed by our team in the context of the same project, as bioguided fractionation of active extracts is indeed a key step to progress towards the development of novel botanical insecticides (Isman and Grieneisen, 2014; Pavela 2015; Pavela et al. 2019).

During the screening, *Piper hispidum* Sw. (Piperaceae) leaves ethyl acetate extract was also identified as active, with LC_{50} values of 70.5 (CI95% 60.4-81.6) and 54.7 (CI95% 45.9-64.0) µg/mL at 24 and 48 h against the laboratory strain Paea. However, due to the vast amount of existing literature on insecticidal Piperaceae and compounds isolated from *Piper* species such as piperine or dillapiole,[42-44] we did not investigate further the chemical composition of this extract (Dorla et al. 2017; Lija-Escaline and al. 2015; Marques and Kaplan 2015). Moreover, if the potential development of a novel botanical insecticide is logically based on its biological activity and its selectivity, other criteria such as the availability of the resource are also fundamental when it comes to valorization (Borges et al. 2019; Pavela et al., 2019). We therefore chose to concentrate on *S. rubra* extract, the most active extract but also the 4th species exploited in the forest industry in French Guiana, leading to wood wastes that could represent a source of valuable material and undisclosed as larvicidal product before the work of our team.

Sextonia rubra (Mez) Van der Werff (Lauraceae) wood and bark extracts were actually shown to possess excellent larvicidal activities in the context of this study. *S. rubra* is a neotropical shade-tolerant rainforest tree species native to South America, and one of the most commercially exploited wood for construction in French Guiana owing to its excellent natural durability. Two compounds rubrynolide and rubrenolide were first isolated from its stem wood in the early ‘70s, and recently characterized *in situ* and identified in bark extracts, and their antifungal and termiticidal activities have been described (Franca et al. 1972; Fu et al. 2018, 2019; Houël et al. 2017; Rodrigues et al. 2010, 2011). As part of the cited work was performed by member of our team, we were able, following the previously described protocols, to characterize the chemical composition of the larvicidal extract and confirm the

presence of the two γ-lactones rubrenolide and rubrynolide. We also highlighted during this project the strong larvicidal activity of these two compounds against *Ae. aegypti*, with respective LC_{50} of 3.84 ± 0.02 and 0.60 ± 0.02 for rubrynolide and rubrenolide at 24 h, and 2.11 ± 0.04 and 0.30 ± 0.02 µg/mL at 48 h, alongside with a measured value of 3.15 ± 0.02 µg/mL for the crude wood extract at 24 h and 2.06 ± 0.02 µg/mL at 48 h (Falkowski et al. 2016). Following a patent deposit concerning the bioactivity of the wood extract and its constituents, a further evaluation of the larvicidal activity and its mechanisms will be performed, alongside with the characterization of its ecotoxicity (Falkowski et al. N° WO2016046489 A1). Cytotoxicity of *S. rubra* was not evaluated in this study on KB and MRC5 cells, however it was demonstrated before that the two major compounds of wood and bark extracts, rubrenolide and rubrynolide, displayed low cytotoxicities on NIH-3T3 mammalian fibroblasts cells with IC_{50} values > 100 µg/mL in each case (Rodrigues et al. 2010). Complementary results have also been published, which highlighted notable toxicity for rubrenolide against several human cancer cell lines (Tofoli et al. 2016). This point will thus be further evaluated in the context of an ongoing project which aims at deepening the above-described results regarding the crude extract and isolated compounds and progressing towards the development of a marketable product.

3.3. Multivariate analysis

A generalized linear model (GLM) regression identified that solvents, organs, family and location are significantly associated to larvicidal potential of the extracts, while light, resources and vegetation type don't (Table 4). A second model was run using only those first four factors and a Tukey HSD test was computed on this second model. The significantly different comparisons are listed in Table 5 and full data are available in Table S2.

Variable	Degree of freedom	Sum of square	Mean of square	F-value	P-value

Solvent	3	30.79	10.264	13.943	1.19e-08
Botanical family	35	46.97	1.342	1.823	0.00367
Location	8	62.24	7.781	10.569	1.88e-13
Light	2	1.32	0.658	0.894	0.40986
Ressource	2	0.26	0.129	0.175	0.83941
Vegetation type	3	3.48	1.162	1.578	0.19420
Plant organs	9	19.57	2.174	2.953	0.00209
Residuals	389	286.37	0.736		

Table 4 Anova analysis on quasi-Poisson generalized linear model (GLM) results

		CI 95%		
	Differences	**Lower**	**Upper**	**P-value**
Solvent [a]				
W / PE	-0.707	-1.046	-0.368	< 0.001
PE / M	0.584	0.283	0.885	< 0.001
W / EA	-0.479	-0.788	-0.171	< 0.001
M / EA	-0.356	-0.622	-0.091	0.003
Organs				
Inflorescence / Aerial parts	1.334	0.229	2.438	0.006
Stems / Inflorescence	-1.234	-2.301	-0.166	0.01
Leaves / Inflorescence	-1.201	-2.259	-0.142	0.013
Fruits / Aerial parts	1.171	0.067	2.276	0.028
Stems / Fruits	-1.072	-2.139	-0.004	0.048
Family				
Lauraceae / Annonaceae	1.704	0.222	3.187	0.006
Lauraceae / Bignoniaceae	1.82	0.021	3.62	0.043
Lauraceae / Costaceae	1.701	0.091	3.31	0.023
Lauraceae / Dilleniaceae	2.411	0.261	4.561	0.009
Lauraceae / Euphorbiaceae	1.8	0.35	3.25	0.001
Lauraceae / Fabaceae	1.697	0.301	3.093	0.002
Loranthaceae / Lauraceae	-1.949	-3.802	-0.096	0.025
Malpighiaceae / Lauraceae	-1.979	-3.513	-0.444	0.001
Malvaceae / Lauraceae	-2.05	-3.973	-0.126	0.02
Meliaceae / Lauraceae	-1.889	-3.645	-0.133	0.018
Sapindaceae / Lauraceae	-1.61	-3.118	-0.101	0.02
Sapotaceae / Lauraceae	-1.93	-3.729	-0.131	0.018

Siparunaceae / Lauraceae	-1.91	-3.709	-0.111	0.022
Solanaceae / Lauraceae	-1.543	-3.064	-0.023	0.041
Location [b]				
Si-Mo	-2.353	-3.328	-1.377	< 0.001
Rg-Mo	-2.454	-3.48	-1.428	< 0.001
Mo-Mc	2.414	1.415	3.413	< 0.001
Mo-Ma	2.391	1.389	3.393	< 0.001
Mo-Ko	2.383	1.393	3.373	< 0.001
Mt-Mo	-2.581	-3.924	-1.238	< 0.001
Rm-Mo	-3.126	-5.251	-1.002	< 0.001
Ro-Mo	-3.898	-6.748	-1.048	0.001

[a] W: water. PE: petroleum ether. M: methanol. EA: ethyl acetate

[b] Si: Sinnamary. Mo: Montsinéry-Tonnegrande. Rg: Régina. Mc: Macouria. Ma: Mana. Ko: Kourou. Mt: Matoury. Rm: Rémire-Montjoly. Ro: Roura

Table 5 Tukey HSD significant pairwise differences between variables

Analysis revealed that mortalities observed for methanol and water extracts were significantly lower than those obtained for petroleum ether and ethyl acetate extracts. Similar results had been previously reported in structure-activity studies (Carreno Otero et al., 2014; Santos et al. 2010). It can be assumed that more polar compounds are less prone to penetrate larvae cuticles, whereas lipophilic compounds have higher affinity for cell membranes and insect cuticles (Chen et al. 2014; Santos et al. 2010). In a study comparing the larvicidal, morphological and behavorial response of *Ae. aegypti* to various extracts of *Argemone mexicana* L. (Papaveraceae), apolar extracts were shown to be the most efficient, inducing modification of larvae cuticles (Warikoo and Kumar, 2013). Our dataset reinforces the interest of lipophilic extracts and compounds as larvicidal products.

Among plant organs (bark, wood, stem, leaves, roots, aerial parts, whole plant, inflorescence and fruits), mortalities induced by the inflorescence extracts were significantly higher than for

aerial parts, stems and leaves. Fruits also induced higher mortality than aerial parts and stems. Inflorescences were only collected from *Costus* species (*C. erythrothyrsus*, *C. spiralis* var. *villosus*, *C*. cf. *spiralis*) and among the 7 tested extracts, 2 exhibited strong larvicidal potential (97-100% mortality) whereas the other 5 were inactive (0-2% mortality). Fruits were collected from 3 species (*Matayba arborescens* and *Cupania scrobitulata*, Sapindaceae, *Tetracera asperula*, Dilleniaceae) and alongside the leaves of *Maytenus* sp. (Celastraceae). Over 9 tested extracts 4 were moderately active (32 to 64% larval mortality) whereas the remaining 5 were inactive (0-1% mortality), including the 2 *Tetracera* extracts (Table S1 Supporting Information). As discussed above, particular cases (damaged *Costus* inflorescence) and chemotaxonomy (Celastraceae and Sapindaceae being well-known insecticidal plants) may explain part of these results. However, the fact that reproductive organs (fruits and inflorescences) exhibit significantly higher larvicidal effect than non-reproductive organs (aerial parts, stems and leaves) is a point of interest in the light of plant species defense. In their work, McCall and Fordyce could not conclude on a possible more intense defense allocation in flowers compared to leaves (McCall and Fordyce 2010). This result possibly originated either from the fact that flowers are not so more valuable than leaves, or simply from a lack of power of the analysis due to a reduced dataset. A recent work for its part demonstrated that wild tobacco flowers accumulate large amounts of defensive compounds, which expression is specifically regulated by jasmonate phytohormones signaling (Li et al. 2017). Moreover, reproductive tissues (including anthers, nectary, ovary, style and stigma) where shown to exhibit higher relative levels of defense-related compounds than vegetative tissues (leaf, root, stem and seed) in a metabolic specialization study of the *Nicotiana attenuata* Torr. ex S. Watson (Solanaceae) model species (Li et al. 2016). Also, the comparison of the natural variation in glucosinolate between vegetative and reproductive tissues in *Boechera stricta* (Graham) Al-Shehbaz (Brassicaceae) revealed much higher concentrations of these defense compounds in fruits compared to leaves (Keith and Mitchell-Olds, 2017). In our case, insecticide activity was detected in inflorescence and fruits extracts of *Costus* and *Cupania* species while leaves and

stem extracts were all inactive. For *Maytenus* and *Matayba* extracts, the observation of the dataset does not lead to obvious conclusions concerning defense allocation. In our study, roots or barks were not highlighted as organ leading to higher proportion of larvicidal extracts. However, these tissues may be of interest in a wider dataset. For example, higher levels of glucosinolate were detected in roots than in shoots of several species, and a higher chemical diversity of both monoterpenes and sesquiterpenes were released by barks compared to leaves immediately after mechanical damage for 178 individual trees belonging to 55 angiosperm species in French Guiana (Courtois et al. 2012; Tsunoda et al. 2017). This later finding was attributed to a larger investment in chemical defenses in the bark. Overall, our data together with the above cited literature suggest that plant organs are differently protected against pests. More active insecticides are found in reproductive organs, and recent herbivory/damage can significantly increase the probability to obtain active extracts. The choice of lipophilic extraction solvent is also critical.

Results concerning families and location are difficult to interpret due to the small size of the dataset. Although expected according to other works, light, resource availability and vegetation type did not significantly affect insecticide potential (Endara and Coley, 2011; Fine et al. 2006, 2013; Smilanich et al. 2016). One reason may be that the objective of developing botanical insecticides prompted us to investigate specifically plant qualitative defense compounds (small weight highly active molecules). These do not represent the totality of plant defensive arsenal. Quantitative defense compounds, such as tannins, are not accessible by these techniques (De Almeida et al. 2005).

4. Conclusion

The above discussed examples distinctly point out the fact, highlighted by Isman and Grieneisen (2014), that collecting a single sample from a single specimen does not allow to conclude on the interest of a given species as a new source of insecticide, and that chemical characterization of the studied extracts can clearly add value to this type of study. Moreover,

we also exemplified that plant defensive chemistry mechanisms are crucial while trying to discover insecticidal products, even if the search for toxic compounds only encompasses a small facet of this highly complex machinery. Multivariate analysis allowed us to identify lipophilic solvents as the most interesting to yield insecticide extracts, and highlighted the fact that extending screening to various plant tissues, in particular reproductive organs, could lead to new promising larvicidal compounds. Analyzing existing dataset and conducting screening studies inspired by the functional role of secondary metabolites in nature, in the light of the chemistry of defense and with the understanding of the mechanisms driving resource allocations as proposed by Berenbaum or Miresmailli and Isman, could therefore help renewing the old-fashioned field of insecticidal natural products (Berenbaum 1995; Miresmailli and Isman 2014).

Supporting information

Supporting information may be found in the online version of this article.

Conflict of interest

On behalf of all authors, the corresponding authors state that there is no conflict of interest.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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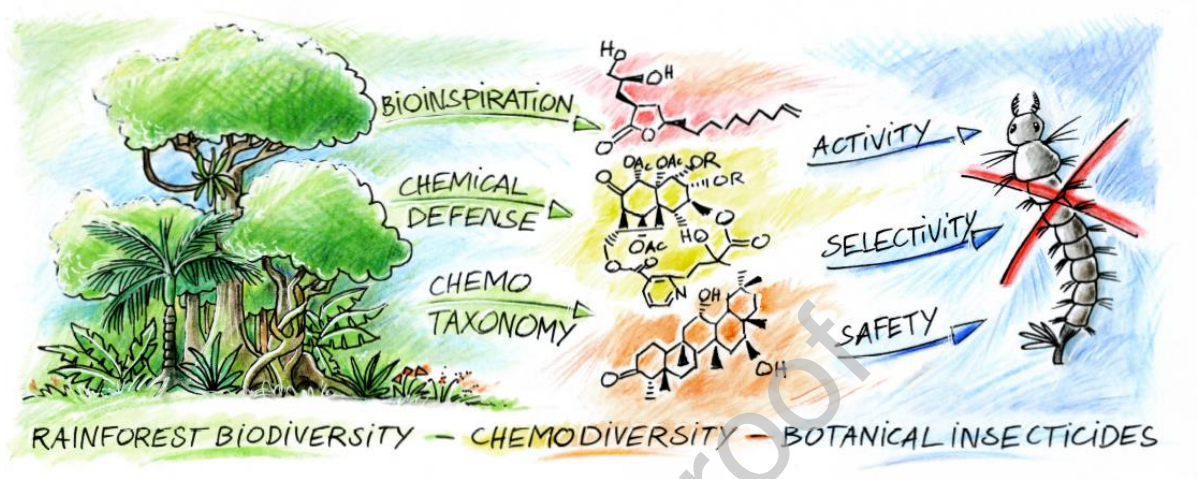
Summary

36 French Guiana biodiversity was explored for the search of novel larvicidal products against

37 both insecticide-susceptible and -resistant *Aedes aegypti* populations.



Graphical abstract