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Plant therapy in the Peruvian Amazon (Loreto) in case of infectious diseases and its antimicrobial evaluation.

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Abstract

Ethnopharmacological relevance

The plant species reported here are used in contemporary phytotherapies by native and neo-urban societies from the Iquitenian surroundings (district of Loreto, Peruvian Amazon) for ailments related to microbial infections. Inhabitants of various ethnic origins were interviewed and 81 selected extracts were evaluated for their antimicrobial properties against a panel of 36 sensitive and multi-resistant bacteria or yeast. Medicinal plant researches in the Peruvian Amazon are now significant, but none of them has focused on an exhaustive listing of identified species tested on so many microbes with standardized experiments (to obtain MIC value).

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Aim of the study

The aim of the study was to inventory the plants used against infections in the Loreto, an Amazonian region of Peru. It led to the new identification of secondary metabolites in two plant species.

Materials and methods

Ethnographic survey was carried out using "participant-observation" methodology and focus on bioprospecting of antimicrobial remedies. Selected plant extracts and antimicrobial drugs were tested *in vitro* with agar dilution method on 35 bacteria strains and 1 yeast to evaluate their Minimal Inhibitory Concentration (MIC). Microdilution methods using 96-well microtiter plates were used for the determination of MIC from isolated compounds, and cytotoxicity in HepG2 cells from some selected extracts were also evaluated. Activity-guided isolation and identification of compounds were performed by various chromatographic methods and structural elucidations were established using HRMS and NMR spectroscopy.

Results

This study outlined antimicrobial activities of 59 plant species from 33 families (72 single plant extracts and 2 fermented preparations), 7 mixtures, and one insect nest extract against 36 microorganisms. Of the 59 species analysed, 12 plants showed relevant antibacterial activity with MIC $\leq$ 0.15 mg/mL for one or several of the 36 micro-organisms (*Aspidosperma excelsum, Brosimum acutifolium, Copaifera paupera, Erythrina amazonica, Hura crepitans, Myrciaria dubia, Ocotea aciphylla, Persea americana, Spondias mombin, Swartzia polyphylla, Virola pavonis, Vismia macrophylla*). Examination by bioautography of *E. amazonica, M. dubia* and *O. aciphylla* extracts allowed the phytochemical characterization of antimicrobial fractions and compounds.

Conclusion

This study suggested an *a posteriori* correlation of the plant extract antimicrobial activity with the chemosensory cues of the drugs and attested that those chemosensory cues may be correlated with the presence of antimicrobial compounds (alkaloids, tannins, saponosids, essential oil, oleoresin…). It also led to the first isolation and identification of three secondary metabolites from *E. amazonica and M. dubia*

Keywords: Antimicrobial activity; Loreto; Peru; Medicinal plant; Traditional use.

1. Introduction

The study took place in the northern region of Peru called Loreto (Amazonian province) with a high level of both biodiversity and traditional knowledge. The bioprospecting was multisite: it was performed in Iquitos surroundings and along the Pastaza river with various quechua-speaking communities (including roughly ten villages from Santa Ana to Sabalo Yacu, see graphical abstract). Mixed-blood native people's ethnomedical practices are part of a larger ethnomedical system including neighboring Amerindian knowledge and practices. This knowledge has been transmitted through constant interactions and exchanges between lowland Amazonian societies (such as quechua-speaking populations from the river Pastaza and Huallaga regions) and mixed-blood urban native populations, leading to a large repository of folk medicine (Gutierrez Choquevilca, 2011; Jauregui et al., 2011; Luna, 1984 and 1986; Sanz-Biset and Cañigueral, 2011).

The plant species reported here as "local phytotherapies" are used by native and neo-urban societies from the district of Loreto (Peruvian Amazon). The Iquitenian riverine populations dwelling the area of study have been engaged in constant communication with native populations, since the "rubber boom" period of the late 19th century. These exchanges occur in particular with the Shipibo from the Ucayali River, the Lowland Quechua people (from the Napo, Tigre, Huallaga and Pastaza river), the Cocama (from the Amazon and Marañon River) and other ethnic groups. For these reasons, ethnomedical knowledge in the area of study stands at the confluence of indigenous and mixed-blood native urban people medicinal practices.

In the tropical rainforest region, infectious diseases are usually the leading cause of human death. In recent years, multiple antibiotic drug resistance has been developed over the word due to indiscriminate use of commercial antimicrobial; therefore, the use of other remedies, as accessible and efficient medicinal plants, with antimicrobial activities is often still the best alternative in "developing" countries (Girish and Satish, 2008).

2. Materials and methods

2.1. Ethnobotanical survey

For this ethnographic survey, a "participant-observation" methodology was adopted and focus put on bioprospecting of antimicrobial remedies. Interviews were performed in two main contexts, such as the Belén Market in Iquitos (where native urban people commercialized medicinal plants), or specific conversation with traditional healers (temporarily present near the city for medical or social reasons). On the other hand, long-time fieldwork was performed in more remote lowland quechua-speaking villages during humanitarian missions (Pastaza, Datem de Marañon). This project was realized in accordance with the Universidad Nacional de la Amazonía Peruana guidelines (Laboratorio de Investigación de Productos Naturales Antiparasitarios de la Amazonia LIPNAA, UNAP)

pertaining to ethnopharmacological studies, and ethical approvals with signature from each informant were obtained before investigations.The ethnobotanical study was conducted during May-September 2014 and 2016. Claims of effective therapy by traditional herbalists for the treatment of infectious diseases or symptoms as cough, diarrhea, abdominal dysfunctions, skin and genito-urinary diseases, conjunctivitis and bronchitis have prompted our interest (see traditional uses, Table 1). These biomedical uses of plant-derived drugs belonged to different categories according to the International Classification of Primary Care (ICPC): mainly infections or parasites but also dermatological and gastrointestinal disorders (Staub et al., 2015).

Information presented here was compiled through informal interviews and rainforest walks with healers, midwives, and local people with knowledge in medicinal plant (around 50 people were interviewed with varied contributions). Taste and smell characterizations of every species were compiled but did not constituted an exhaustive experimental data collection for a complete correlation “organoleption to bioactivity”.

Our survey led to collect and identify 59 plants (and 1 insect nest) for their anti-infectious use and to assess their antimicrobial activity. The minimal inhibitory concentration (MIC) was determined *in vitro* against 36 microbial strains belonging to 24 different species.

2.2. Plant material

The plants collected were vouchered, deposited and identified at the Herbarium of the Universidad Nacional de la Amazonia Peruana by the botanist Juan Celidonio Ruiz Macedo, (UNAP, Iquitos) and according to regional floras (Duke and Vasquez, 1994; Rutter, 1990; Velasquez, 1990).

Plant samples were dried for two days, or one week for fresh fruits, at 45 °C, finely ground with a hammer mill, and extracted overnight with methanol using gentle shaking at room temperature (10 g of powdered plant in 250 mL of MeOH). The processing of the plants performed in this study was different from the traditional preparations (Table 1). Therefore, it is not an exact replication of the traditional knowledge but it is admitted that methanolic plant extracts provide more consistent antimicrobial activity (Murphy Cowan, 1999). Moreover, methanol provides a more complete extraction, including fewer polar compounds, and is more representative of the traditional preparations than other chemical solvents. The extracts were filtered through filter paper, dried under reduced pressure at 40°C. Nonetheless, some traditional preparations (see additional information of Table 1) were non-reproductible in laboratories, which explained discordances between the expected efficacy of a traditional remedy and the evaluation of its activity *in vitro*.

Resins were expressed, dried and partially dissolved in methanol, but their low solubility in medium culture could explain their low and unrepresentative activity.

Furthermore, the most cited plant combinations against skin infections (here called “APCT” for Amasisa, Papailla, Capirona and/or Tangarana: *Erythrina amazonica, Momordica charantia, Calycophyllum spruceanum, Triplaris peruviana* respectively) were tested. The aim was to evaluate the antimicrobial activity and potential synergy of these four

164 species in various mixtures (A+P, A+C, A+T, P+T, C+T, P+C, A+P+C+T with fixed
165 concentrations).
166

167 2.3. Antimicrobial tests

168

169 *Methodology and data analysis*

170

171 It must be considered that indigenous diagnosis is inherently relative, due to cultural
172 reasons. Indeed, according to nosological folk theories, indigenous people more frequently
173 identify and focus on symptoms (fever, cold, tiredness, diarrhea…) and cultural concept of
174 illness rather than on specific biological aetiologies such as microbes or parasites (Roumy et
175 al. 2007). Consequently, any specific identified "bacteria" were ascribed to a so-called
176 "traditional use". Therefore, plant extracts were tested on a large panel of 36 bacteria or yeast,
177 in order to be more representative of the infectious pathogens enhanced in the described
178 disease.
179 Control antibiotics had been previously performed and were available for each strain
180 (Table 2), but it is important to remind that antibiotics are pure active compounds that do not
181 have a vegetal origin, therefore it cannot be expected that plant extracts could have the same
182 efficiency as antibiotics (Das and Tiwari, 2010). Nevertheless, those tests were realized to
183 give useful information for the choice of plant remedies against infectious diseases.
184 Correlations between characteristics of plants and antibacterial activity were compared
185 with Chi-square or Fisher's tests. Categorical variables were characterized by absolute
186 numbers and percentages. Analyses were conducted using SAS software (SAS version 9.4,
187 SAS Institute Inc., Cary, NC, USA) (Fig. 1).
188

189 *Selected microorganisms*

190

191 Plant extracts were tested on a large panel of 36 bacteria or yeast. The agar dilution
192 method (macrodilution) with methanolic extracts enabled to test antimicrobial activity without
193 the problem of solubility (except for some resins), to obtain standardized numeric value
194 (MIC) for every microorganism.
195 Antimicrobial activity of these vegetal species was evaluated for the first time against a panel
196 of 36 pathogenic and multiresistant microbs which, in most cases, have been recently isolated
197 from human infections. For comparison, reference strains from the American Type Culture
198 Collection (ATCC) were included. The selected pathogens can be involved in diseases cited
199 by the informants, and nosocomial or opportunistic infections; this diversity permitted to
200 extend the scope of therapeutic applications.
201 The investigations used standardized methodology with internationally recognized protocols
202 (CLSI, 2006). The various microorganisms (12 Gram-positive, 21 Gram-negative, 3
203 miscellaneous) were all able to grow aerobically in Mueller Hinton Agar (MHA) media. The
204 different strains selected were:

Enterobacteria lactose-positive and VP negative (which are usually poorly resistant and pathogenic): *Escherichia coli* (8138: penicillin resistance; ATCC25922: NA); *Citrobacter freundii* (11041; 11042: cephalosporin resistance).
Enterobacteria lactose-positive and VP positive (with high antibiotic resistance): *Klebsiella pneumoniae* (11016; 11017: penicillin resistance), *Enterobacter cloacae* (11050; 11051: cephalosporin resistance; 11053: NDM-1), *Enterobacter aerogenes* (9004: BLSE) and *Serratia marcescens* (11056; 11057: cephalosporin resistance).
Enterobacteria lactose-negative (more pathogenic than previous lactose-positive Enterobacteria): *Proteus mirabilis* (11060), *Providencia stuartii* (11038), *Salmonella* sp (11033).
Gram-positive cocci (involved in some external infections described by informants): *Staphylococcus aureus* (8146: meticillin- and kanamycin- resistant; 8147), *Staphylococcus epidermidis* (5001, 10282, 8157), *Staphylococcus lugdunensis* (T26A3), *Staphylococcus warneri* (T12A12), *Enterococcus* sp (8153: erythromycin- and clindamycin-resistant), *Enterococcus faecalis* (C159-6 vancomycin-susceptible), *Streptococcus agalactiae* (T25-7, T53C2), *Streptococcus dysgalactiae* (T46C14).
Gram-negative bacteria (also found in nosocomial infections): *Pseudomonas aeruginosa* (8131; ATCC 27583), *Acinetobacter baumanii* (9010: VEB-1; 9011: multiresistant), *Stenotrophomonas maltophilia* (TP 2012), *Yersinia pseudotuberculosis* (2777).
Miscellaneous microorganisms: *Mycobacterium smegmatis* (acid-alcohol resistant bacillus 5003, a fast growing bacterium but not equivalent to *Mycobacterium tuberculosis*), *Corynebacterium striatum* (Gram-positive bacillus: T25-17) and the yeast *Candida albicans* (10286) also potentially engaged in various local diseases (Table 1).

Minimal inhibitory concentration (MIC) determination

MIC (Minimal Inhibitory Concentration) determinations of crude extracts were carried out using the agar dilution method stipulated by the CLSI agar dilution methods (CLSI, 2006). The MIC of the extracts and standards for antibiotics were determined for 35 bacterial strains and 1 yeast by diluting the extracts in Mueller Hinton Agar (MHA) media. Crude extracts were dissolved in methanol to reach a final concentration of 12 mg/mL for activity tests.

The inhibitory concentrations were ranged between 0.07 and 1.2 mg/mL in five dilutions (1.2, 0.6, 0.3, 0.15 and 0.07 mg/mL); 0.07 mg/mL was considered as the lowest concentration for a preliminary screening. According to previous publications about the anti-infective potential of natural products, plant extract were considered as active at MIC ≤ 0.15 mg/mL (Cos et al., 2006; Rios and Recio, 2005). Petri dishes, were inoculated with strains (10^4 CFU, obtained by dilution in Brain heart, BH) using a Steer's replicator and were incubated at 37ºC for 24 h. MIC was defined as the lowest concentration of extract without bacterial growth after incubation. The extracts with MIC ≤ 1.2 mg/mL were tested in triplicate at lower concentrations (mean absolute deviation is done for values: 1.2 ± 0.4; 0.6 ± 0.2; 0.3 ± 0.1; 0.15 ± 0.05; 0.07 ± 0.03). The standards (Gentamicin, Vancomycin, Amoxicillin and Amphotericin B) were tested in triplicate in 12 concentrations ranged between 0.03 and 64 mg/L (Table 2). MIC determinations of the pure compounds were realized by broth

microdilution method with serial dilution using 96-well microtiter plates against sensitive bacteria *Staphylococcus epidermidis* (5001). Five concentrations of each compound, from 1.2 to 0.07 mg/mL were used. They were serially twofold diluted with Ringer's Cysteine solution (RC) in five wells. Two wells were represented as bacteria culture control (positive control) and medium sterility control (negative control). Then the wells were loaded with Mueller Hinton medium (MH) and bacterial suspension (10^4 bacteria/mL), giving a final volume of 200 μL. The plates were incubated overnight at 37 °C and bacterial growth was indicated by direct spray of 0.2 mg/mL *p*-iodonitrotetrazolium (INT).

TLC and Bioautography

Plates (Silica gel 60 Xtra Sil G/UV254) were developed with ethyl acetate: methanol (1:1), used for methanolic extracts from *Erythrina amazonica*, *Myrciaria dubia and Ocotea aciphylla*. Organic compounds on TLC were revealed with 254-365 nm UV light or anisaldehyde sulphuric acid reagent (tannins were revealed with $FeCl_3$ aqueous solution, triterpenes with Liebermann Burchard reactant, and alkaloids with Dragendorff reagent).
TLC plates for bioautography were dried and over laid by nutrient agar seeded with an overnight culture of *Staphylococcus epidermidis* (Gram +, 5001). The plates were incubated for 24 h at 37 °C and then sprayed with a solution of *p*-iodonitrotetrazolium violet (one hour later, clear zones appeared corresponding to bacterial inhibition).

2.4. Cytotoxicity in HepG2 cells

Methanolic dried extracts of *Erythrina amazonica*, *Myrciaria dubia* and *Ocotea aciphylla* were suspended in DMSO to obtain an original concentration of 150 mg/mL.
Cells were subcultured in 96-well plates with 3500 cells/well in 100 µL of growth medium (Dulbecco's Modified Eagle Medium, DMEM) supplemented with 10% foetal bovine serum 50 U/mL penicillin and 50 µg/mL streptomycin)). After 24 h of incubation (in humidified atmosphere, 5% CO2 at 37 °C), the medium was discarded and replaced with 5 dilutions of the plant extracts in DMEM (at 300, 150, 75, 32 and 16 µg/mL) in quadruplicate. Cellular growth control was performed using medium with 0.2% DMSO. After an incubation period of 48 h, the medium was discarded and replaced with DMEM containing 0.5 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). After 1 h 30 of incubation at 37°C in the 5% CO_2 incubator, the water-insoluble formazan was dissolved in 100 µL dimethyl sulfoxide and the absorbance was measured at a test wavelength of 550 nm using a UV-spectrometer (Tecan, Spark 10M). The cytotoxicity of the crude extracts or standard (camptothecin: IC_{50}: 0.9 ± 0.1 µg/mL) was determined by comparing the absorbance of cells treated by extracts with absorbance of control cells cultured in 0.2% DMSO. Data were expressed as percentages of inhibition calculated according to the formula (% Cell viability = Abs cells with extract x 100 / Abs cells control) and analyzed by linear regression.

2.5. Compound isolation and identification

General

NMR spectra were recorded on a Bruker AVANCE 500 spectrometer (1H at 500 MHz- and 13C-NMR) in methanol-d4 or chloroform-d6. HRMS analyses were carried out in positive or negative mode, using a Thermo Fisher Scientific Exactive Orbitrap mass spectrometer equipped with an electrospray ion source.

Erythrina amazonica alkaloids isolation

Air-dried and powdered bark (500 g) was extracted three times overnight with methanol (1 L) using gentle shaking at room temperature (3 x 1 h). Dried crude extract (37 g) was suspended in MeOH (400 mL) and precipitated at low temperature (12 h at 5°C). After centrifugation, solid phase (9.8 g without alkaloids) was put aside and supernatant (30.2 g, containing alkaloids) was evaporated and chromatographed on silica gel column eluted with successive eluent Tol/EtOAc then EtOAc /MeOH gradients. Fractionations were monitored by TLC with visualization under UV (254 and 365 nm) and revealed with Dragendorff reagent, leading to 6 fractions (A to F). Fraction D (1.1 g) was separated by centrifugal partition chromatography: CPC with a tertiary system (EtOAc/MeOH/H_2O; 1:1:0.3; v/v/v) in ascending mode (8 mL/min during 30 min), then extrusion with stationary phase was realised (30 mL/min during 10 min). CPC was performed using a 250 mL rotor (SCPC-250-L, Armen Instrument) and a Shimadzu Prominence LC-20AP binary pump. Fractions were collected every minute and 10 subfractions (1 to 10) were then pooled according to TLC analysis. Fraction number 9 (providing by extrusion) was purified on preparative silica TLC (eluted by Hex/MeOH; 45:55; V/V) to obtain 9 mg of compound **1**.

Erysotrine **1**: yellow amorpheous solid (9 mg), identified by NMR and MS spectra (See supplementary data). HRESIMS: *m/z* $[M+H]^+$ 314.1762 (calcd for $C_{19}H_{24}NO_3$, 314.1751). Spectral data were consistent with those of Sarragiotto (Sarragiotto et al., 1981).

Myrciaria dubia methanolic extracts from leave, seed and peel

600 g of leaves were extracted according previous process (used for *E. amazonica*) to obtain 65 g of dried powder. Then, crude extract was subjected to column chromatography on silica gel and sequentially eluted with *n*-hexane, ethyl-acetate and methanol. Fractionation was monitored by TLC (with visualization under UV at 254 and 365 nm, and revelation with Liebermann Burchard or anisaldehyde sulphuric acid reagent) to afford 13 fractions (F1-F13). Fractions F6 and F8 were further purified by silica gel medium-pressure column chromatography and preparative TLC using mixtures of *n*-Hex/EtOAc/MeOH of increasing polarity, leading to the isolation and identification by NMR spectroscopy of *β*-sitosterol (13 mg) and betulinic acid (11 mg).

Further chromatography of methanolic seed and peel extracts by UHPLC-Mass spectrometry permitted to highlight the presence of betulinic acid in these drugs (see supplementary data).

Ultra-High Performance Liquid Chromatography (UHPLC) analyses were carried out using an Acquity UPLC H-Class Waters system equipped with a diode array detector (DAD) and an Acquity QDa ESI-Quadrupole Mass Spectrometer.

The stationary phase was a Waters Acquity BEH C18 column (2.1x50 mm, 1.7 µm). The mobile phase consisted of 0.1% formic acid in water or in acetonitrile, at 0.45 mL/min, 30 °C. The gradient of acetonitrile was: 15% (0-1 min), 15-98% (1-7 min), 98% (7-9 min). The ionization was performed in negative mode. Cone voltage was set at 15 V with a capillary voltage at 0.8 kV and probe temperature at 600 °C.

3. Results

3.1. Ethnobotanical study

Ethnomedical context

The medicinal plant market of Belén (Iquitos) started in the late sixties and the majority of the "*puestos*" (around 40 stalls in total) are concentrated in one single street called "pasaje paquito". The daily rent for each stall is ≈ 10 soles ≈ 3-4 €. Stalls are under the responsibility of women engaged in the commercialization of medicinal plants to urban population and to shamans from different native origins (Cocama, Lowland Quechua, Shipibo…). The majority of the approximately 150 commercialized plant species come from distant forest areas (River Napo, River Nanay and rural surroundings of the road Iquitos-Nauta) (Galy et al., 2000). Plants are sold at a low price (1-3 soles per kg of bark for popular recipes) and their commercialization is supposed to contribute to the local progressive disappearance of spontaneous species such as *Tynanthus panurensis*, *Maytenus macrocarpa*, *Hymenaea oblongifolia*. A set of other species with specific ritual use like *Banisteriopsis caapi, Brugmansia* spp*., Dieffenbachia* spp*., Jatropha* spp. and/or easy to grow species, are currently cultivated in private gardens and fields.

Another part of the fieldwork was performed in lowland quechua-speaking villages from the Pastaza River (located at 400 km in East of Iquitos) where medical and traditional knowledge are characterized by quite different nosological classifications and aetiological categories of illnesses, even if there is an increasing cultural influence of the national society (Roumy et al., 2007).

Around 50 persons were interviewed with varied contributions, depending on their willingness and availability (half an hour to few days), that is the reason why numbers of citations were not statistically considered in this study. Nonetheless, social origins of informants were very different (inhabitant of indigenous community, shaman, old woman or teenager) providing the collection of exhaustive data from the studied area.

The results of this study are summarized in Table 1.

Table 1: Alphabetic list and traditional anti-infectious uses of the investigated species.

***Scientific name*, (Family), voucher specimen**	**Usual vernacular name**	**Part used**	**Traditional uses to treat infectious diseases (organoleptic properties: *sm*: smell, *ta*: strong taste, *st=sm+ta, and* Ø)**
Abelmoschus moschatus Medik. (Malvaceae), 25336	Mishu isma	Fr, S	Seeds macerated one hour in water (or urine) with edible powdered cucurbitaceous seeds and drunk by children as expectorant against pulmonary infections. (S: *st*) Decoction of entire fruit used to wash intimate areas. (Fr: **Ø**).
Abuta grandifolia (Mart.) Sandwith (Menispermaceae), 40880	Abuta hembra	B	Decoction used to clean sores, or drunk against pulmonary infections, dengue and malaria (overdosage can lead to blindness, *ta*).
Alchornea castaneifolia (Humb. & Bonpl. ex Willd.) A. Juss. (Euphorbiaceae), 33679	Ipururo	B, L	Alcoholic maceration ("x raices"). Decoction mixed with tobacco juice and applied on skin mycosis. (**Ø**).
Aristolochia iquitensis O.C. Schmidt. (Aristolochiaceae), 40708	Lengua de perro	AP	Crushed and directly applied on skin infections. (*sm*).
Artocarpus altilis (Parkinson) Fosberg. (Moraceae), 35784	Pan del arbol	B, Re	Decoction of bark applied on skin infections and resin ointment for bone fractures or hernia. (*ta*).
Aspidosperma excelsum Benth. (Apocynaceae), 37478	Remocaspi	B	Decoction of bark drunk against malaria mixed with *C. odorata* for intestinal infections or external use against herpes. (*st*).
Brosimum acutifolium Huber. (Moraceae), 39422	Murure, tamamuri	B	Alcoholic maceration ("x raices"). (*sm*).
Calycophyllum spruceanum (Benth.) Hook. (Rubiaceae), 28182	Capirona	B	Decoction of bark applied on pimples and cold sores, also in mixture APCT*. (*sm*).
Campsiandra angustifolia Spruce ex Benth. (Fabaceae), 36287	Huacapurana	B	Alcoholic maceration ("x raices"). (*sm*).
Capsicum annuum L. (Solanaceae), 37872	Macusari	Fr+S	Decoction mixed with tobacco juice against skin inflammations, or mixed with *C. trinitatis* for shamanic use against sorcery aggression. (*ta*).
Cariniana decandra Ducke. (Lecythidaceae), 28022	Tahuari	B	Alcoholic maceration as "x raices", for general purification or skin infections. (*sm*).
Cecropia ficifolia Warb. ex Snethl. (Urticaceae), 39552	Cetico	L	Juice of crushed young leaves applied against conjunctivitis. (**Ø**).
Cedrela odorata L. (Meliaceae), 033958	Cedro rojo	B	Bark decoction mixed with *A.excelsum* against malaria and intestinal infections. (*st*).
Ceiba pentandra (L.) Gaertn. (Malvaceae), 33496	Lupuna blanca	B	Shamanic use in sorcery with reputation of symbolic poison. (*sm*).
Chenopodium ambrosioides L. (Amaranthaceae), 32480	Païco	AP	Decoction with *S. mombin*, *Malachra ruderalis*, *Plantago major*, *H. crepitans* with salt against sores and epidermal mycosis (external use). Crushed leaves ingested against intestinal parasites. (*sm*).
Cissus ulmifolia (Baker) Planch. (Vitaceae), 39079	Sapohuasca	AP	Alcoholic or aqueous maceration of leaves drunk against hernia or intestinal disorder. (*sm*).
Clusia amazonica Planch. & Triana. (Clusiaceae), 35928	Renaquilla	B + R, Re	Crushed and directly applied on skin infections. Alcoholic maceration ("x raices"), and reinforcement in post-partum or hernia. (*ta*).
Copaifera paupera (Herzog) Dwyer (Fabaceae), 33420	Copaiba	Re	2 drops in hot water with *Uncaria tomentosa* drunk against gastritis, or mixed with *J. curcas* against pulmonary infections. External application against venereal diseases. (*ta*).
Cornutia odorata (Poepp.) Poepp. ex Schau (Lamiaceae), 21637	Sacha mukura	AP	Decoction drunk against respiratory infections with fever. (*st*).
Couroupita guianensis Aubl. (Lecythidaceae), 033949	Ayahuma	Fr	Ablution against skin infections due to insect bites, plague or disinfectant for animals. Decoction against digestive infections. Shamanic use in sorcery. (*st*).
Crescentia cujete L. (Bignoniaceae), 33852	Huingo	Fr	Decoction with *G. americana* and termites nest (*Isoptera* spp), concentrated and drunk against pulmonary infections. Decoction with *A. excelsum* against malaria. (*ta*).
Croton trinitatis Millsp. (Euphorbiaceae), 40990	Catahuio macho	L	Decoction of leaves or burnt stems applied on infected sores. (*sm*).
Dracontium spruceanum (Schott) G.H.Zhu (Araceae), 38014	Jergon sacha	T	Pulverized dried tuber applied on sore or snake bit. (*sm*).
Eleutherine bulbosa (Mill.) Urb. (Iridaceae), 38423	Yahuar piri-piri	T	Crushed and diluted in tepid water, drunk against fever or bloody diarrhea. Pulverized dried bulb applied on sore or ablution with the juice. (*st*).
Erythrina amazonica Krukoff. (Fabaceae), 037513	Amasisa	B	Decoction of barkused to wash skin infections (dermatitis, herpes, vaginitis …) or mixed in APCT*. Alcoholic maceration with *M. alliacea* as external or oral administrations. (*sm*).
Euterpe precatoria Mart. (Arecaceae), 26315	Huasaï	R	Decoction drunk as depurative and against malaria. (**Ø**).
Ficus insipida Willd. (Moraceae), 33854	Ojè	Re	Decoction of resin is drunk as violent purgative against intestinal parasites (can be harmful). External ointment for cutaneous leishmaniasis (mixed with *S. mombin*). (*ta*).
Genipa americana L. (Rubiaceae), 33811	Huito	Fr, Re	Decoction with *C. cujete* and termites nest (*Isoptera* spp), concentrated and drunk against pulmonary infections. (*ta*).
Grias neuberthii J.F. Macbr. (Lecythidaceae), 33652	Sacha mangua	B, Fr, S	Pulverized seed or concentrated decoction applied in the nostril against sinusitis. Edible fruit and depurative. (Fr: *st*, S: *ta*). Decoction of bark drunk against malaria. (B: *st*).
Hura crepitans L. (Euphorbiaceae), 35787	Catahua	B, Re	Mixed in decoction (cf. *C. ambrosioides*), or applied as plaster with *M. esculenta* against venereal diseases. Shamanic use in sorcery (caustic resin that must be used diluted). (*st*).
Hymenaea oblongifolia Huber. (Fabaceae), 39395	Azucar huaio	B	Alcoholic maceration ("x raices"). (*sm*).

Jatropha curcas L. (Euphorbiaceae), 35786	Piñon blanco	L, S	Pulverized seeds used as disinfectant and purgative. Infusion of leaves against mouth infections. Shamanic use in sorcery. (*sm*).
Jatropha gossypifolia L. (Euphorbiaceae), 033699	Piñon rojo, negro	L, S	Similar to *J. curcas*, but more "powerful". (**Ø**).
Lantana trifolia L. (Verbenaceae), 39054	Tunchi albahaca	L	Decoction applied on infected sores (confused with "Catahuio embra"). (*st*).
Lonchocarpus nicou (Aubl.) DC. (Fabaceae), 26307	Barbasco blanco	L, R	Drug crushed, boiled and reduced for external ointment against infections. Plaster of leaves on thorax against tuberculosis. (*ta*).
Macrolobium acaciifolium (Benth.) Benth. (Fabaceae), 27401	Habilla	S	Pulverized seed in hot water drunk as cathartic or against stomach disorder (toxic, imported from Lima). (**Ø**).
Manihot esculenta Crantz (Euphorbiaceae), 37823	Yuka, masato	T	Starchy substance used for plaster and against skin inflammation or fever. Crushed and fermented with saliva and water to obtain a drink called "masato". Basic food and beverage. (**Ø**).
Mansoa alliacea (Lam.) A.H. Gentry (Bignoniaceae), 33856	Ajo sacha	R, L	Ablution with crushed leaves to cure rheumatisms, bones pain, inflammations (can be drunk but need special diets and gradual increasing dosage). (*sm*).
Maquira coriacea (H. Karst.) C.C. Berg (Moraceae), 37553	Capinuri	B, L, Re, Br	Resin diluted in tepid water or decoctions, drink against hernia, prostatitis and abdominal dysfunction. (*st*).
Maytenus macrocarpa (Ruiz & Pav.) Briq. (Celastraceae), 36978	Chuchuhuasi	B	Alcoholic maceration ("x raices"). (*st*).
Momordica charantia L. (Cucurbitaceae), 39508	Papaïlla	AP	Decoction to wash skin infections (dermatitis, herpes, pimple, scab…) and plaster with *M. esculenta*. Decoction mixed in "APCT*" for external or oral administrations. (*sm*).
Musa L. (Musaceae), 32124	Agua de guineo negro, inguiri	Re	Fresh exudates from the cut stem of banana tree drunk against tuberculosis; it can be mixed with *C. cujetes*, *G. americana* and termites nest (*Isoptera* spp). Also mixed with resin (*C. paupera*, *Croton lechleri*) against gastritis and syphilis. Directly applied on cutaneous inflammation and infections. (**Ø**).
Myrciaria dubia (Kunth) McVaugh (Myrtaceae), 32508	Camu-camu	BFr, L, S	Fruit juice drunk as tonic against common cold. *(sm).* Bark in alcoholic maceration ("x raices", *ta*) against common cold and arthritis (may be mixed with leave decoction, *ta*).
Ocotea aciphylla (Nees & Mart.) Mez (Lauraceae), 40930	Doctor caspi	B	Decoction or alcoholic macerate, can be mixed with numerous other remedies (*S. mombin*, *Unonopsis floribunda*, *M. coriacea*, *C. paupera*, "x raices" …) against dental caries, bloody diarrhea, abdominal disorder, vaginal cyst…(*st*).
Persea americana (var. Hass) Mill. (Lauraceae), 33683	Palta	S	Decoction of the pulverized stone to wash intimate areas against gonorrhea. In abortive mixture. (*ta*).
Petiveria alliacea L. (Phytolaccaceae), 35371	Mucura	AP	Crushed leaves with *Ocimun basilicum* applied on conjunctivitis and rinsed with *E. precatoria* in decoction. (*sm*).
Solanum mammosum L. (Solanaceae), 26646	Teta de vaca	Fr	Fruit juice and pulp or dried decoction applied on cutaneous mycosis and scab. (*ta*).
Spondias mombin L. (Anacardiaceae), 33691	Ubos colorado	B	Leaf or bark plaster as antiseptic and aqueous preparation drunk against diarrhea and genital infections or sores (leishmania…). (*st*).
Stachytarpheta cayennensis (Rich.) Vahl (Verbenaceae), 033882	Verbena negra, sacha verbena	AP	Infusion against diarrhea. (*st*).
Strychnos sp L. (Loganiaceae), 33876	Camalonga	S	Alcoholic maceration with onion and garlic against pulmonary infection. Shamanic use in sorcery (toxic). (*st*).
Swartzia polyphylla DC. (Fabaceae), 24608	Cumaceba	B	Alcoholic maceration as "x raices". (*sm*).
Tessaria integrifolia Ruiz & Pav. (Asteraceae), 39481	Pajaro bobo	Br	Decoction against kidney and liver inflammations. (*st*).
Triplaris peruviana Fisch. & Meyer ex C.A. Meyer (Polygonaceae), 20092	Tangarana	B	Decoction applied on pimples and cold sores, also in mixture APCT* against skin infections. (*sm*).
Tynanthus panurensis (Bureau) Sandwith (Bignoniaceae), 37571	Clavo huasca	B	Alcoholic maceration as "x raices". (*sm*).
Verbena litoralis Kunth (Verbenaceae), 39133	Verbena blanca	AP	Crushed leaves or juice applied on foot mycosis, can be mixed with *Malachra ruderalis* against itching. Infusion with "*A. excelsum* or *P. alliacea*" against malaria and diarrhea. (*st*).
Virola pavonis (A. DC.) A.C. Sm. (Myristicaceae), 33890	Cumal blanco	B, R	Root and stem bark decoction against skin infection and mouth mycosis. (*sm*).
Vismia macrophylla Kunth Hypericaceae	Pichirina	B, L, Re, S	Powdered drug or resin are applied externally, for treating fungal infections (B, L, Re: *st*)
Xanthosoma violaceum Schott (Araceae), 28445	Patiquina negra	L	Decoction as disinfectant and plaster. Shamanic use to cure sorcery aggression. (*sm*).
Zygia latifolia (L.) Fawc. & Rendle (Fabaceae), 39199	Yutsu	B, L	Pulverized bark or decoction of leaves drunk against fever and malaria. (B: *b,* L: **Ø**).
Erythrina amazonica, Momordica charantia, Calycophyllum spruceanum, Triplaris peruviana	APCT*: Amasisa + Papailla + Capirona + Tangarana	B AP B B	Usual mixture of bark and herbs as decoction to wash epidermal infections (dermatitis, herpes, scab, vaginitis …). (*st*).

Sceliphron sp. (Sphecidae)	Nido de avispa	Nest	Alcoholic concentrated maceration mixed with camphor applied against mumps. Aqueous maceration with *Malachra ruderalis* ("malva") and salt to obtain a plaster against skin inflammation and mycosis. (**Ø**).

AP: aerial parts, B: stem bark, BFr: bark of fruit, Br: branch, Fr: fruit, L: leaves, R: root, Re: resin, T: tuber, APCT*: mixture of Amasisa, Papailla, Capirona and/or Tangarana (disinfectant); "x raices": mixture of barks usually prepared as a tonic alcoholic maceration. Organoleptic properties: *sm*: smell, *ta*: strong taste, *st=sm+ta, and* Ø without salient taste or flavor. Plant species that have not been tested in this study (reported in the table with their entire scientific names) were previously evaluated with similar conditions (Roumy et al., 2015).

Additional information on several species and preparation from Table 1 are reported here:

- "x raices": called "7 or 21 raices" ("raices" meaning "roots") are mixtures of barks (genuine preparations were constituted of roots, but now, they are widely prepared with barks which are easier to collect). Those preparations are usually used as "tonic", to reinforce bodily functions and libido or against rheumatism.
- The beverage prepared with *Manihot esculenta* called *masato* was obtained by fermentation of the crushed tuber in water in presence of *Saccharomyces cerevisiae* var. *bayanus* (1%) during 3 and 7 days at 30 °C in laboratory, then filtered extracts were dried and tested *in vitro.*
- Adulterations were observed for some species: *tunchi albahaca* (*Lantana trifolia*) sold as *catahuio embra* for replacement under the same vernacular name because the original species could not be provided at this time. It has also been reported that some vernacular names, such as *sapohuasca* and *amasisa* could refer to different species (*Cissus ulmifolia* or *C. sicyoides* and *Erythrina amazonica* or *E. fusca*).
- Some specific traditional uses could not be evaluated with "*in vitro"* experimental conditions e.g.:

  "Toma de pacto": "drink with deal"; the patient promises something to the plant spirit that he must realize after ingestion of the plant preparation. In case of non-achievement, the person can be injured by the plant spirit (uses of *Aristolochia iquitensis*, *Xanthosoma violaceum*, *Ceiba pentandra*).

  "Agua de tiempo": means "water at any time", refers to a plant extract that can be drunk at any time during the day instead of any other liquids, so it may imply a wide variation of the posology, e.g.: *Crescentia cujete*, *Genipa Americana, Musa* sp.

  "Serenar": means "to alleviate" in Spanish and "lay down" in regional quechua: this consists of leaving aqueous plant extract outdoors during the whole night under the light of the moon and in contact with the morning dew (e.g. preparations of *Bixa orellana, Momordica charantia, Dracontium spruceanum*).

  "Baño de florecimiento": means "bath for symbolic blossoming", administered to heal or purify the body from misfortune or injury (called "saladera"), it is a superficial bath or an exposure to the vapor of a plant decoction (e.g. use of *Xanthosoma violaceum* with *Hura crepitans* and *Jatropha gossypifolia*). These rituals of "washing" or "purifying" the body had a symbolic efficacy within their cultural context, but these plants were not really employed as disinfectant agents even if some of them contained a little bit of antibacterial essential oil as *J. gossypifolia* leaves (e.o.: 0.03 - 0.32 %; Reis et al., 2013; Sunday et al., 2016).
- Quechua language and culture have an important influence on plant taxonomies and usually refer to the organoleptic properties of vegetal species, such as color, shape and odor. For

instance, *Ayahuma* means "black head" and refers to the "dark" properties of this round fruit in sorcery (*Couroupita guianensis*); *Mishu isma,* means "cat's stool" and refers to the musky smell of *Abelmoschus moschatus* seeds. The same influence of organoleptic characteristic is observed for Spanish denominations: e.g. *Ubos colorado* for "colorful grape" from *Spondias mombin* tree. These examples illustrated the important influence of the organoleptic properties on plant identifications or uses as previously described (Geck, et al, 2017; Bennett, 2007), as well as their mnemonic functions: "Plants that are both empirically effective and easy to remember are more likely to be retained in oral traditions" (Shepard, 2004).

3.2. Results of biological tests

Results constituted, with similar previous data (Roumy et al, 2015), a contribution to the "materia medica for infectious diseases in the Amazonian district of Loreto" (Heinrich, 2018). The evaluation of antimicrobial activities of the 59 plant species (33 families, 72 simple extracts, 7 mixtures and 2 fermented preparations), and one insect nest extract (*Sceliphron* sp.) showed antimicrobial activities (MIC $\leq$ 0.15 mg/mL) for 14 plant extracts among 72 (14/72 = 19.4 %), mainly composed of barks (8/14 = 57.1 %).

The 12 other species characterized by higher MIC (0.15 < MIC $\leq$ 0.3 mg/mL) were mostly composed of barks (10/12 = 83.3 %) and always characterized by an important taste or/and smell, but were not considered as active in this study for phytochemical screening. Nonetheless their MIC corroborated the trend observed for higher antimicrobial activity in case of bark with chemosensory cues ($p = 0.0001$ and $p = 0.01$; See table 2).

The plant extract antibacterial activities were not systematically correlated to the route of administration (external or internal), nor correlated to the traditional indications (category of disease, pain localization, aetiology, toxic or sacred species) or the frequency of plant citations. Moreover, traditional uses of the investigated species (Table 1) indicated that a lot of remedies were prepared as mixtures of different plants ("x raices"), where compounds possibly enhance or complement each other. Few bioassays were performed in this study with the mixture of 4 plant species (APCT*: *Calycophyllum spruceanum, Erythrina amazonica, Momordica charantia, Triplaris peruviana*) but did not exhibited synergistic variation of activity (only phenomena of additivity were observed).

The three plant species selected for chemical study, *Erythrina amazonica*, *Myrciaria dubia* and *Ocotea aciphylla*, were also characterized by a low cytotoxicity in HepG2 cells (IC_{50} > 0.3 mg/mL), demonstrating a specific antibacterial activity.

4. Discussion

4.1. Antimicrobial activities and organoleptic drugs properties

The analysis of results revealed different levels of activity according to the part of the plant tested, in favour of barks, and attested that none of the drug without organoleptic cues

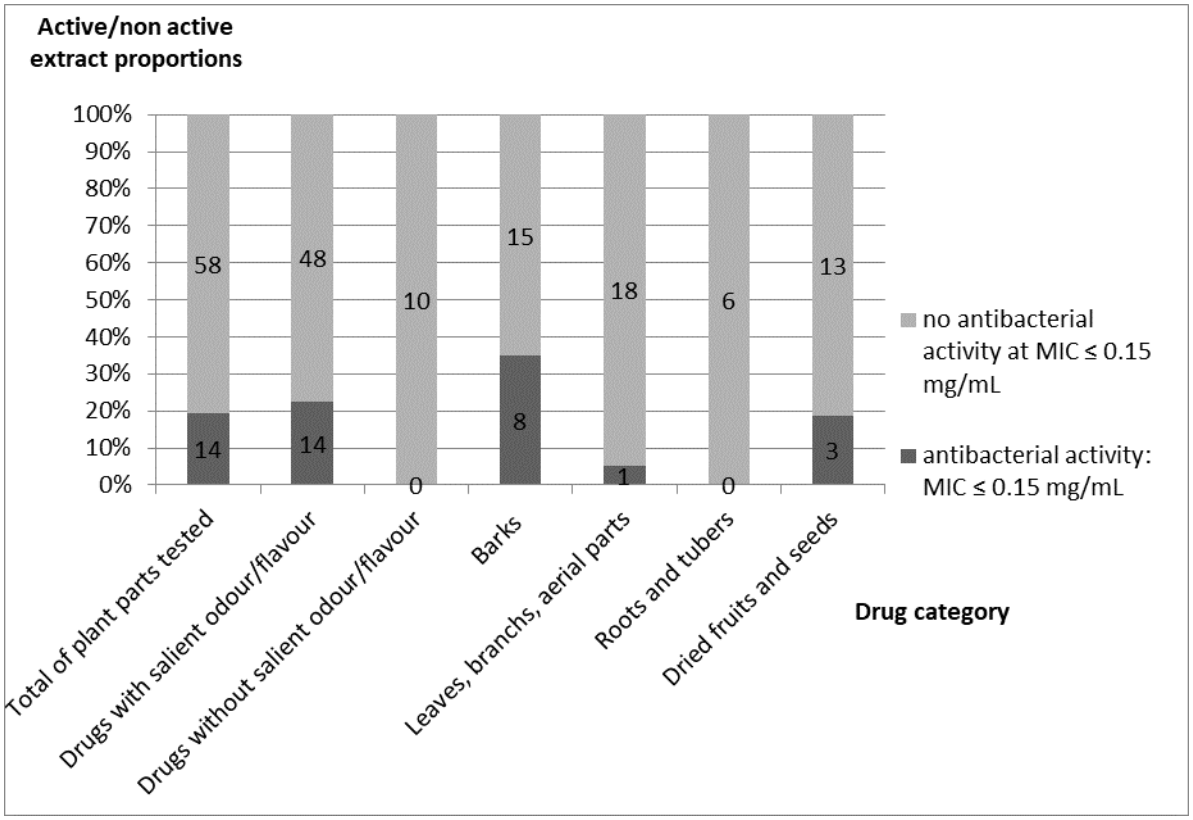
had antimicrobial activity with MIC ≤ 0.3 µg/mL. These results may be correlated to a higher content of tannin or a best stability of essential oil in these rough drugs, explaining the antimicrobial activity and the sensory cues (bitter or fragrant). These observations may be enlighted by 2 different ways:

First of all, in the Amazonian pharmacopeias, organoleptic properties are excellent guides for selecting or memorizing medicinal plants (Leonti et al., 2001) and plants that are both empirically effective and easy to remember are more likely to be retained in oral traditions (Shepard, 2004). That may explain why species, which had both chemosensory properties and therapeutic activity, are more transmitted than others.

Secondly, in modern medicine and phytotherapy, substances like essential oils, terpenoids, alkaloids, or tannins have already been studied for their antimicrobial activities and are usually characterized by strong taste and/or flavor (essential oils are aromatic, alkaloids are bitter, tannins are astringent…). In fact, antibacterial derivatives from plants are usually strong tasting and/or aromatic (Lai and Roy, 2004; Rios and Recio, 2005).

Nonetheless, it must be pointed out that lot of plants without smell or taste are also used in local phytotherapies but usually with other indications (e.g. bonne fractures, sprain, hernia).

Fig. 1. Distribution of antimicrobial activity by drug category



The trend observed for antimicrobial activity in case of bark with chemosensory cues were statistically significant for MIC ≤0.3 mg/mL (chi-square test: p = 0.0001 and p = 0.01, see Table 2), but not for MIC ≤0.15 mg/mL (p = 0.26 and p = 0.19)

4.2. Antimicrobial plant spectra and phytochemicals

Interpretation of antimicrobial plant spectra showed more relevant activity against Gram-positive (G+) and miscellaneous strains. Nonetheless, some of these extracts were also powerful against Gram-negative bacteria (G-), including species containing oleo-resin, essential oil or latex usually composed of terpenoids (Zacchino et al., 1998). These lipophilic compounds are involved in bacterial membrane disruption and can be found in some of the botanical families tested in this study: Anacardiaceae, Euphorbiaceae, Lauraceae, Lecythidaceae, Meliaceae, Moraceae, Myristicaceae, Myrtaceae and Verbenaceae. Furthermore, species with more specific activity against G+ bacteria did not contain essential oil but alkaloids (*Aspidosperma* spp*, Erythrina* spp*, Abuta* spp) or lipids (*Persea americana* seed, Giffoni Leite et al., 2009). Those observations were likely to be the result of the differences in cell wall structure between G+ and G- bacteria since the Gram-negative outer membrane containing lipopolysaccharides acts as a barrier to many environmental substances, including antibiotics. Moreover, the strains susceptibility to the antibacterial activity of a same extract did not depend on their sensibility or resistance to the tested antibiotics (see Table 2), which is in favor of very different - thus readily complementary - modes of action.

All those results and discussions were in accordance with previous studies assessed on 40 other Peruvian plant species with similar microbiological methods (Roumy et al., 2015).

A search in the literature indicated that the potential antibacterial agents of some of the investigated plants had already been characterized. Among the 12 species characterised by a relevant antibacterial activity (MIC $\leq$ 0.15 mg/mL), 8 bark extracts were brought out: *Aspidosperma excelsum* (with indole monoterpenoid alkaloids as antimicrobial compounds: Correia et al., 2008), *Brosimum acutifolium* (flavonoids and lignoids as active compounds, *ibid.*), *Erythrina amazonica* (flavonoids, pterocarpan and erythrinan alkaloids as antibacterial compounds identified in the genus *Erythrina*: Wanjala et al., 2002, Yenesew et al., 2005, De Avila et al., 2018), *Hura crepitans* (alkaloids, flavonoids and tannins: Oloyed and Olatinwo, 2014), *Ocotea aciphylla* (neolignans: Felicio, et al., 1986; gallic tannins and aromatic compounds revealed in this study), *Spondias mombin* (tannins, flavonoids, anthraquinones and saponins: Ayoka et al., 2008), *Swartzia polyphylla* (flavonoids, pterocarpan and saponins: Osawa et al., 1992), *Vismia macrophylla* (bark or leaves composed of essential oil with γ-bisabolene and cytotoxic anthrone derivatives, Buitrago et al., 2015; Hussein et al., 2003). Relevant activity was also observed for *Copaifera paupera* (resin: diterpenes then lipids and tannins; Arruda et al., 2019; Correia et al., 2008), *Persea americana* (seed containing antibacterial phenolic compounds and lipids: Sidrim et al., 2009), *Myrciaria dubia* (seed or fruit bark with tannin and phloroglucinol as antibacterial compounds, Myoda et al. 2010; Kaneshima et al., 2017) and *Virola pavonis* (root, neolignans and other phenylpropanoids derivatives: Ferri and Barata, 1992; Zacchino et al., 1998),. Bibliographic research on barks species from *Erythrina amazonica* and *Ocotea aciphylla* or leaves from *Myrciaria dubia* did not allow identification of their antimicrobial content, justifying here their chemical study (Ueda et al., 2004).

4.3. Activity-guided isolation of antimicrobial compounds

Examination by bioautography of *E. amazonica, M. dubia* and *O. aciphylla* extracts allowed the phytochemical characterization of antimicrobial fractions and compounds as described below.

Bioautography of *E. amazonica* methanolic and dichloromethane bark extracts revealed an antibacterial activity due to alkaloids and lipophilic compounds. The fractionation and purification of the alkaloid by chromatography on silica gel columns, CPC then preparative TLC, led to the identification by NMR spectroscopy of erysotrine (MIC=1.2 mg/mL against *Staphylococcus epidermidis* 5001). This compound was here identified for the first time in *E. amazonica* species.

Besides acylphloroglucinols and other polyphenols derivatives with antibacterial properties (Borges et al., 2014; Kaneshima et al., 2017), bioautography of methanolic extracts from *Myrciaria dubia* seed, peel and leaf, showed triterpenic apolar active compounds revealed by Liebermann Burchard reactant. Successive silica gel column chromatography from leaf extract permitted the purification and isolation of two compounds identified by NMR spectroscopy as *β*-sitosterol (MIC=1.2 mg/mL) and betulinic acid (inactive at 1.2 mg/mL on *Staphylococcus epidermidis* 5001). *β*-sitosterol was identified for the first time in

this plant species, and betulinic acid was isolated before from *M. dubia* seed but not from a leaf or peel.

Lastly, evaluation of antibacterial activity of methanolic and dichloromethane extracts from *Ocotea aciphylla* bark supported by bioautography (on S. *epidermidis* 5001) revealed the presence of polar and aromatics active compounds. TLC analysis also showed the presence of inactive hydrolysable tannins, and no activity was observed for the chloromethylenic extract. Therefore, analysis concluded to the presence of antibacterial lipophilic aromatic compounds from methanolic extract but did not allowed their identification.

5. Conclusion

Interpretation of antimicrobial spectra showed inhibitory activity (14/72 plant extracts with MIC $\leq$ 0.15 mg/mL) against pathogens that were not necessarily conventionally incriminated with the diseases described by the informant. The main therapeutic indications collected for these drugs were the venereal, cutaneous, or digestive infections whereas the most sensitive pathogens were the *Staphylococcus* spp. mainly responsible for mucocutaneous infections**.**Those data and previous discussions prove that traditional indication is not exclusively linked to a specific *in vitro* antibacterial activity of the remedy.

The phytochemical study led to the isolation and identification for the first time of the erysotrine, an erythrinan alkaloid from *E. amazonica* bark*,* and the triterpenic compounds: betulinic acid and *β*-sitosterol from *M. dubia* peel and leaf.

Single antibacterial and antifungal *in vitro* assays were not fully reliable for determining plants efficacy against infection. In particular, it was inappropriate for plants used in case of infectious disease as anti-inflammatory, analgesic, antiparasitic, antiviral or potentiator agents. (Bussmann et al., 2008). Nevertheless, the results of this study support to a certain degree the traditional medicinal uses of the plants evaluated, and highlighted the relevance of our ethnobotanical approach. Further biological assays on plant mixtures activity and plant toxicity will be performed to improve scientific interpretation of this traditional knowledge.

The study also exhibited the need to collaborate with traditional healers to improve the recognition of their medical practices (choice of plant prescription), and the value of their tools and medicines. Even if biological test just revealed a part of remedies therapeutic value, an insight of indigenous and mixed-blood native people worldviews may contribute to a better understanding of their traditional medical system and its relation to biological efficacy.

Conflict of interest

The authors declare that they have no conflict of interest.

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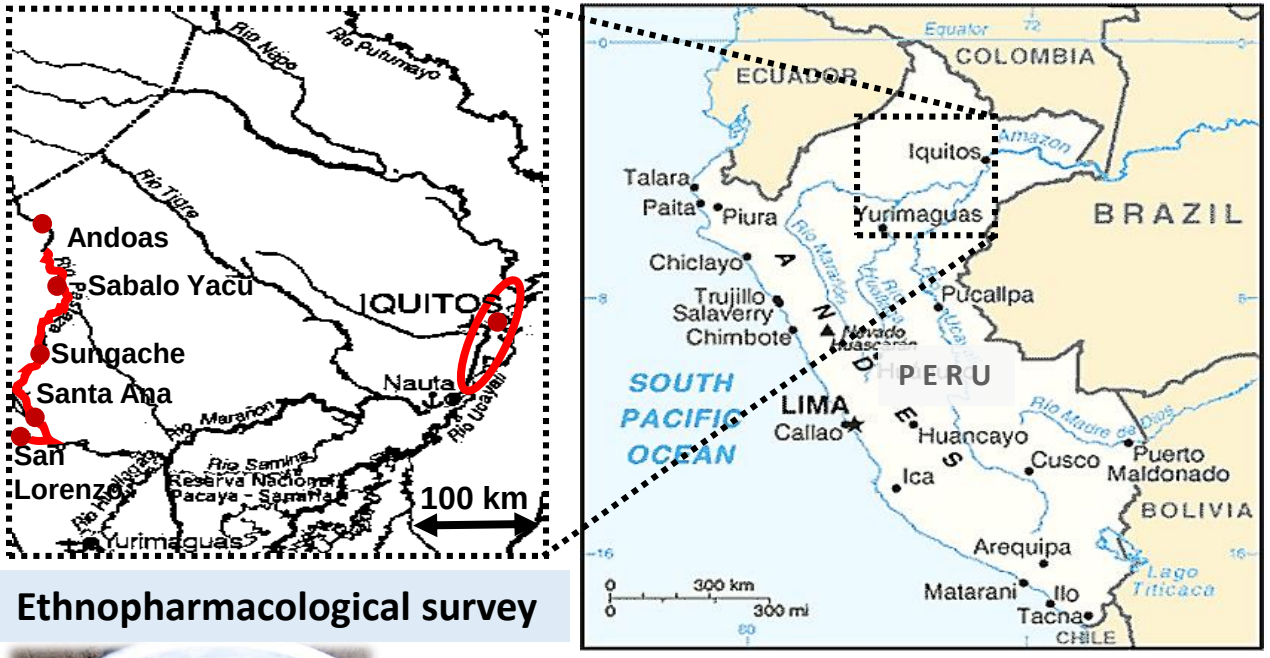
Plants / Microbiological strains			Gram-negative bacilli (enterobacteria)																Gram-negative bacilli (non enterobacteria)					Gram-positive cocci												Miscellaneous strains		
Family	***Species***	**Part**	***Citrobacter freundii***		***Escherichia Coli***		***Eenterobacter aerogenes***	***Enterobacter cloacae***			***Kelbsiella pneumoniae***	***Serratia marcescens***		***Proteus mirabilis***	***Providencia stuartii***	***Salmonella sp.***	***Yersinia pseudotuberculosis***		***Acinetobacter baumanii***		***Pseudomonas aeruginosa***		***Stenotrophomas maltophilia***	***Enterococcus faecalis***	***Enterococcus sp.***	***Staphylococcus aureus***		***Staphylococcus epidermidis***			***Staphylococcus lugdunensis***	***Staphylococcus warneri***	***Streptococcus agalactiae***		***Streptococcus dysgalactiae***	***Corynebacterium striatum***	***Mycobacterium smegmatis***	***Candida albicans***
			11041	**11042**	**8138**	**ATCC25922**	**9004**	**11050**	**11051**	**11053**	**11016**	**11017**	**11056**	**11057**	**11060**	**11038**	**11033**	**2777**	**9010**	**9011**	**8131**	**ATCC 27583**	**TP2012**	**T25-17**	**8153**	**8146**	**8147**	**8157**	**10282**	**5001**	**T26A3**	**T12A12**	**T25-7**	**T53C2**	**T46C14**	**T25-17**	**5003**	**10286**
Anacardiaceae	*Spondias mombin*	B	1.2	1.2	-	-	-	-	-	-	-	0.6	-	-	0.6	0.6	-	0.3	0.6	-	-	0.3	0.3	0.3	-	1.2	1.2	0.15	0.6	1.2	0.3	0.6	-	-	1.2	0.3	0.3	-
Apocynaceae	*Aspidosperma excelsum*	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	1.2	0.6	0.6	0.15	0.15	0.3	0.15	0.07	0.3	0.6	0.6	0.6	0.3	1.2
Asteraceae	*Tessaria integrifolia*	Br	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	0.6	-	-	-	-	-	-	-
Celastraceae	*Maytenus macrocarpa*	B	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	1.2	-	-	-	1.2	1.2	-	-	1.2	1.2	0.6	1.2	-	1.2	1.2	1.2	1.2	1.2	1.2	1.2	-
Clusiaceae	*Clusia amazonica*	B + R	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-	-	-	-	1.2	0.6	-	1.2	0.6	0.6	0.3	0.6	1.2	0.6	0.6	0.6	0.6	0.6	0.6	0.6	1.2
Cucurbitaceae	*Momordica charantia*	AP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1.2	-	-	1.2	1.2	0.6	1.2	0.6	-	0.6	0.6	0.3	1.2	-	-
Cyperaceae	*Scleria macrophylla*	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-
Euphorbiaceae	*Alchornea castaneifolia*	L	-	-	-	-	-	-	-	-	-	0.6	-	-	-	-	-	-	-	-	-	0.6	0.6	-	-	-	1.2	0.6	1.2	-	0.6	1.2	-	-	-	-	1.2	-
	Croton trinitatis	L	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	0.6	-	-	1.2	0.6	1.2	1.2	-	-	-	1.2	1.2	-	1.2	1.2	-	-	1.2	1.2	1.2	-
	Hura crepitans	B	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-	-	-	-	0.6	0.6	-	0.6	0.6	0.6	0.15	0.3	0.6	0.15	0.6	0.3	0.3	0.15	0.3	0.3	0.6
	Jatropha curcas	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1.2	-	-	1.2	1.2	0.6	0.6	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	-
Fabaceae	*Campsiandra angustifolia*	B	-	-	-	-	-	-	-	-	-	0.6	-	-	-	-	-	0.6	-	-	1.2	0.6	0.6	1.2	1.2	0.6	0.6	0.3	0.6	1.2	0.6	0.6	0.6	0.6	0.6	0.6	0.6	1.2
	Copaifera paupera	Re	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	1.2	0.6	-	0.6	0.3	0.15	0.3	-	-
	Erythrina amazonica	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	0.3	0.3	0.3	0.15	0.3	0.15	0.3	0.15	0.3	0.15	0.15	0.3	-
	Hymenaea oblongifolia	B	-	-	-	-	-	-	-	-	-	0.6	-	-	1.2	1.2	-	-	-	-	-	0.6	0.6	-	-	1.2	0.6	0.3	0.6	-	0.6	0.6	1.2	1.2	0.6	0.6	0.6	-
	Lonchocarpus nicou	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-
	Swartzia polyphylla	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	1.2	-	-	0.6	0.6	0.6	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.15	0.15	0.3	0.3	0.6
	Zygia latifolia	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	-	-	1.2	1.2	-	1.2	0.6	0.6	0.3	0.6	1.2	0.6	1.2	1.2	1.2	0.6	0.6	0.6	1.2
Hypericaceae	*Vismia macrophylla*	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	1.2	0.15	0.15	0.15	0.15	0.15	0.15	0.3	0.6	0.6	0.3	0.15	0.15	1.2
		L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	1.2	-	1.2	-	0.3	0.15	0.3	0.3	0.3	0.3	1.2	-	-	0.3	0.3	0.3	-
Iridaceae	*Eleutherine bulbosa*	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	1.2	-	-	0.6	0.6	0.3	0.6	0.6	0.3	0.6	0.3	0.3	0.6	1.2	0.6	1.2

Lamiaceae	*Cornutia odorata*	AP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1.2	-	-	1.2	1.2	-	1.2	-	1.2	0.6	1.2	-	0.6	1.2	1.2	-
Lauraceae	*Ocotea aciphylla*	B	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.3	1.2	1.2	0.3	0.6	1.2	0.6	0.6	0.6	0.6	0.3	0.3	1.2	-	0.6	0.6	0.15	0.6	0.6	0.3	0.6	1.2	0.6	1.2	1.2	0.3	1.2
	Persea americana (var. Hass)	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15	0.3	-	0.15	0.07	0.07	0.15	0.07	0.15	0.07	0.07	0.07	0.15	0.15	0.3
Lecythidaceae	*Cariniana decandra*	B	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-	-	-	-	1.2	1.2	-	-	-	-	1.2	-	-	1.2	1.2	-	-	-	1.2	1.2	-
	Grias neuberthii	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1.2	-	-	0.3	0.3	-	-	1.2	-	0.6	0.6	-	0.6	1.2	-	-	-	1.2	0.6	-
	Ceiba pentandra	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	0.6	-	-	-	1.2	0.6	-
Meliaceae	*Cedrela Odorata*	B	-	-	-	-	-	-	-	-	-	1.2	-	-	1.2	0.6	-	0.6	1.2	-	-	0.6	0.6	-	-	0.6	0.6	0.3	0.6	1.2	0.6	0.6	1.2	1.2	1.2	0.6	0.6	-
Menispermaceae	*Abuta grandifolia*	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	1.2	1.2	0.6	0.6	1.2	1.2	0.6	0.6	1.2	-
Moraceae	*Artocarpus altilis*	B	-	-	-	-	-	-	-	-	-	1.2	-	-	1.2	1.2	-	1.2	-	-	-	1.2	0.6	0.6	0.6	0.6	0.6	0.3	0.3	0.6	0.3	0.6	0.6	0.3	0.15	0.6	0.6	-
	Brosimum acutifolium	B	1.2	1.2	-	-	1.2	1.2	1.2	1.2	-	0.3	1.2	1.2	0.6	0.6	-	0.3	0.6	0.6	0.6	0.15	0.15	-	-	0.3	0.3	0.15	0.15	0.6	0.15	0.15	1.2	1.2	0.6	0.15	0.15	-
Myristicaceae	*Virola pavonis*	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	1.2	0.6	-	-	-	0.6	0.15	0.3	1.2	0.3	0.6	1.2	1.2	0.6	0.6	0.3	-
		B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	1.2	-	0.6	-	-	-	1.2	0.3	0.6	-
Myrtaceae	*Myrciaria dubia*	BFr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	0.3	0.3	-	-	0.15	0.15	0.3	0.3	0.3	-	-	-	0.3	0.3	0.3
		L	-	-	-	-	-	-	-	-	-	1.2	-	-	1.2	-	-	-	-	-	-	-	-	1.2	-	-	-	1.2	-	-	-	-	-	-	-	1.2	1.2	1.2
		S	-	-	-	-	-	-	-	-	-	0.6	-	-	1.2	-	-	1.2	-	-	-	1.2	0.3	0.3	0.3	-	-	0.15	0.3	0.3	0.3	0.3	-	-	-	0.6	0.3	0.3
Polygonaceae	*Triplaris peruviana*	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	1.2	1.2	1.2	1.2	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.3	0.3	0.3	1.2
Rubiaceae	*Genipa americana*	Fr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1.2	-	-	-	1.2	1.2	-	-	1.2	-	-	-	-	-	-	-
Verbenaceae	*Lantana trifolia*	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Stachytarpheta cayennensis	AP	-	-	-	-	-	-	-	-	-	1.2	-	-	1.2	1.2	-	1.2	1.2	-	-	1.2	0.6	-	-	1.2	1.2	0.6	0.6	-	0.6	1.2	-	-	-	1.2	1.2	-
	Verbena litoralis	AP	-	-	-	-	-	-	-	-	-	0.6	-	-	1.2	1.2	-	1.2	-	-	-	0.3	0.3	-	-	1.2	1.2	0.3	0.3	-	0.3	0.6	-	-	1.2	1.2	0.6	-
Vitaceae	*Cissus ulmifolia*	AP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1.2	0.6	-	-	-
Plant mixture	*E. amazonica* + *M. charantia* + *C. spruceanum* + *T. peruviana*		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	1.2	1.2	1.2	1.2	1.2	-
	M. charantia + *E. amazonica*		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1.2	0.6	0.6	-	0.6	-	1.2	1.2	0.6	0.15	1.2	-
	M. charantia + *T. peruviana*		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1.2	0.6	1.2	-	1.2	1.2	1.2	1.2	0.6	0.6	1.2	-
	M. charantia + *C. spruceanum*		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	1.2	1.2	1.2	1.2	-	-
	C. spruceanum + *T. peruviana*		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1.2	0.6	1.2	-	1.2	1.2	1.2	1.2	1.2	1.2	1.2	-
	C. spruceanum + *E. amazonica*		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	1.2	-	-	-	1.2	1.2	0.6	0.6	1.2	-
	E. amazonica + *T. peruviana*		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1.2	1.2	1.2	0.3	0.6	1.2	1.2	1.2	1.2	1.2	0.6	0.6	1.2	-
ANTIBIOTICS (mg/L)	Gentamicin	-	0.25	0.12	0.5	0.5	0.5	0.25	0.25	0.25	0.25	8	0.5	R	0.5	0.25	0.25	0.5	0.25	0.5	1	2	4	2	2	0.5	0.5	2	16	0.25	0.5	0.06	0.5	0.25	0.5	0.06	0.03	na
	Vancomycin	-	0.5	16	na	na	na	4	16	4	na	na	0.5	16	na	4	na	1	16	16	na	na	16	0.5	4	16	1	32	32	0.5	4	4	0.25	0.5	16	0.5	0.5	na
	Amoxicillin	-	1	32	na	16	na	4	2	32	na	na	32	4	na	16	2	4	0.5	32	na	na	2	64	8	32	16	2	na	1	1	1	0.03	0.5	0.5	0.5	1	na
	Amphotericin B	-	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	0.06

AP: aerial parts. B: stem bark and branch. EP: entire plant. Fl: flower. Fr: fruit. L: leaves. R: root. Re: resin. T: tuber, BFr: bark of fruit. MIC (mg/L) of positive controls: nt: not tested na: not active at 64 mg/L. Gentamicin. S: ≤4. R: >8; Vancomycin. S: ≤4. R: >16; Amoxicillin. S: ≤4. R: >16; Amphotericin B. S: ≤1. R: >4. S: sensitive. I: intermediate. R: resistant, Standard deviation for values: 1.2 ± 0.4; 0.6 ± 0.2; 0.3 ± 0.1; 0.15 ± 0.05; 0.07 ± 0.03).

Color code for extracts with MIC< 0.15 mg/mL

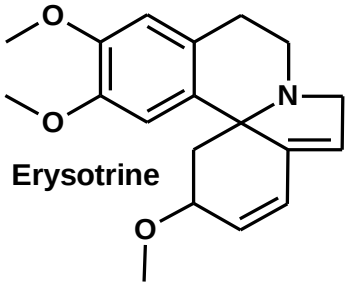
Table 2: Antimicrobial activity of active plant extracts (MIC: mg/mL)



Ethnopharmacological survey

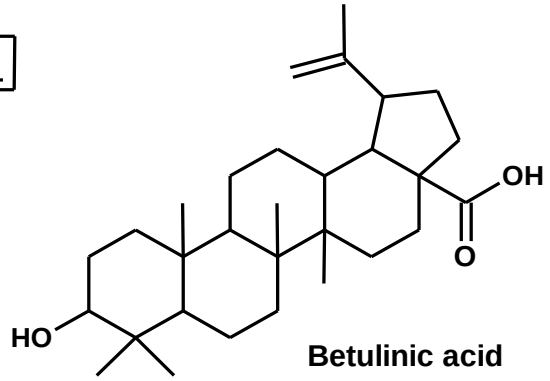
1

Isolation and identification of compounds



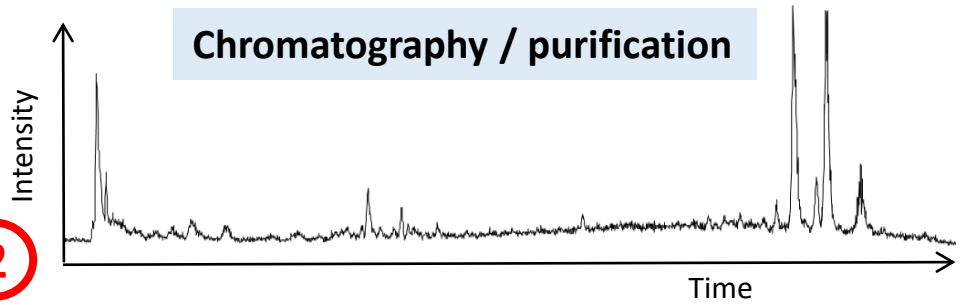
Erysotrine

3

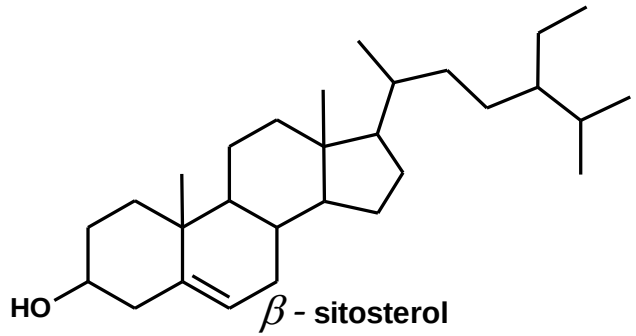


Betulinic acid

Chromatography / purification



2



β - sitosterol

Plant extraction

Antimicrobial tests