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

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

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31 **Abstract**

32

33 ***Ethnopharmacological relevance***

34 The plant species reported here are used in contemporary phytotherapies by native and neo-  
35 urban societies from the Iquitenian surroundings (district of Loreto, Peruvian Amazon) for  
36 ailments related to microbial infections. Inhabitants of various ethnic origins were interviewed  
37 and 81 selected extracts were evaluated for their antimicrobial properties against a panel of 36  
38 sensitive and multi-resistant bacteria or yeast. Medicinal plant researches in the Peruvian  
39 Amazon are now significant, but none of them has focused on an exhaustive listing of  
40 identified species tested on so many microbes with standardized experiments (to obtain MIC  
41 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.

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## 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 Cañigüeral, 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 19<sup>th</sup> 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 world 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)

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

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

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

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## 140 2.2. Plant material

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

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

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

160 Furthermore, the most cited plant combinations against skin infections (here called  
161 “APCT” for Amasisa, Papaila, Capirona and/or Tangarana: *Erythrina amazonica*,  
162 *Momordica charantia*, *Calycophyllum spruceanum*, *Triplaris peruviana* respectively) were  
163 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).

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### 167 2.3. Antimicrobial tests

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#### 169 *Methodology and data analysis*

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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 microbes 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:

205 Enterobacteria lactose-positive and VP negative (which are usually poorly resistant and  
 206 pathogenic): *Escherichia coli* (8138: penicillin resistance; ATCC25922: NA); *Citrobacter*  
 207 *freundii* (11041; 11042: cephalosporin resistance).

208 Enterobacteria lactose-positive and VP positive (with high antibiotic resistance): *Klebsiella*  
 209 *pneumoniae* (11016; 11017: penicillin resistance), *Enterobacter cloacae* (11050; 11051:  
 210 cephalosporin resistance; 11053: NDM-1), *Enterobacter aerogenes* (9004: BLSE) and  
 211 *Serratia marcescens* (11056; 11057: cephalosporin resistance).

212 Enterobacteria lactose-negative (more pathogenic than previous lactose-positive  
 213 Enterobacteria): *Proteus mirabilis* (11060), *Providencia stuartii* (11038), *Salmonella* sp  
 214 (11033).

215 Gram-positive cocci (involved in some external infections described by informants):  
 216 *Staphylococcus aureus* (8146: meticillin- and kanamycin- resistant; 8147), *Staphylococcus*  
 217 *epidermidis* (5001, 10282, 8157), *Staphylococcus lugdunensis* (T26A3), *Staphylococcus*  
 218 *warneri* (T12A12), *Enterococcus* sp (8153: erythromycin- and clindamycin-resistant),  
 219 *Enterococcus faecalis* (C159-6 vancomycin-susceptible), *Streptococcus agalactiae* (T25-7,  
 220 T53C2), *Streptococcus dysgalactiae* (T46C14).

221 Gram-negative bacteria (also found in nosocomial infections): *Pseudomonas aeruginosa*  
 222 (8131; ATCC 27583), *Acinetobacter baumannii* (9010: VEB-1; 9011: multiresistant),  
 223 *Stenotrophomonas maltophilia* (TP 2012), *Yersinia pseudotuberculosis* (2777).

224 Miscellaneous microorganisms: *Mycobacterium smegmatis* (acid-alcohol resistant bacillus  
 225 5003, a fast growing bacterium but not equivalent to *Mycobacterium tuberculosis*),  
 226 *Corynebacterium striatum* (Gram-positive bacillus: T25-17) and the yeast *Candida albicans*  
 227 (10286) also potentially engaged in various local diseases (Table 1).

228

#### 229 *Minimal inhibitory concentration (MIC) determination*

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231 MIC (Minimal Inhibitory Concentration) determinations of crude extracts were carried  
 232 out using the agar dilution method stipulated by the CLSI agar dilution methods (CLSI,  
 233 2006). The MIC of the extracts and standards for antibiotics were determined for 35 bacterial  
 234 strains and 1 yeast by diluting the extracts in Mueller Hinton Agar (MHA) media. Crude  
 235 extracts were dissolved in methanol to reach a final concentration of 12 mg/mL for activity  
 236 tests.

237 The inhibitory concentrations were ranged between 0.07 and 1.2 mg/mL in five  
 238 dilutions (1.2, 0.6, 0.3, 0.15 and 0.07 mg/mL); 0.07 mg/mL was considered as the lowest  
 239 concentration for a preliminary screening. According to previous publications about the anti-  
 240 infective potential of natural products, plant extract were considered as active at MIC  $\leq$  0.15  
 241 mg/mL (Cos et al., 2006; Rios and Recio, 2005). Petri dishes, were inoculated with strains  
 242 ( $10^4$  CFU, obtained by dilution in Brain heart, BH) using a Steer's replicator and were  
 243 incubated at 37°C for 24 h. MIC was defined as the lowest concentration of extract without  
 244 bacterial growth after incubation. The extracts with MIC  $\leq$  1.2 mg/mL were tested in triplicate  
 245 at lower concentrations (mean absolute deviation is done for values:  $1.2 \pm 0.4$ ;  $0.6 \pm 0.2$ ;  $0.3 \pm$   
 246  $0.1$ ;  $0.15 \pm 0.05$ ;  $0.07 \pm 0.03$ ). The standards (Gentamicin, Vancomycin, Amoxicillin and  
 247 Amphotericin B) were tested in triplicate in 12 concentrations ranged between 0.03 and 64  
 248 mg/L (Table 2). MIC determinations of the pure compounds were realized by broth

249 microdilution method with serial dilution using 96-well microtiter plates against sensitive  
250 bacteria *Staphylococcus epidermidis* (5001). Five concentrations of each compound, from 1.2  
251 to 0.07 mg/mL were used. They were serially twofold diluted with Ringer's Cysteine solution  
252 (RC) in five wells. Two wells were represented as bacteria culture control (positive control)  
253 and medium sterility control (negative control). Then the wells were loaded with Mueller  
254 Hinton medium (MH) and bacterial suspension ( $10^4$  bacteria/mL), giving a final volume of  
255 200  $\mu$ L. The plates were incubated overnight at 37 °C and bacterial growth was indicated by  
256 direct spray of 0.2 mg/mL *p*-iodonitrotetrazolium (INT).

257

#### 258 *TLC and Bioautography*

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260 Plates (Silica gel 60 Xtra Sil G/UV254) were developed with ethyl acetate: methanol  
261 (1:1), used for methanolic extracts from *Erythrina amazonica*, *Myrciaria dubia* and *Ocotea*  
262 *aciphylla*. Organic compounds on TLC were revealed with 254-365 nm UV light or  
263 anisaldehyde sulphuric acid reagent (tannins were revealed with FeCl<sub>3</sub> aqueous solution,  
264 triterpenes with Liebermann Burchard reactant, and alkaloids with Dragendorff reagent).

265 TLC plates for bioautography were dried and over laid by nutrient agar seeded with an  
266 overnight culture of *Staphylococcus epidermidis* (Gram +, 5001). The plates were incubated  
267 for 24 h at 37 °C and then sprayed with a solution of *p*-iodonitrotetrazolium violet (one hour  
268 later, clear zones appeared corresponding to bacterial inhibition).

269

#### 270 2.4. Cytotoxicity in HepG2 cells

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272 Methanolic dried extracts of *Erythrina amazonica*, *Myrciaria dubia* and *Ocotea*  
273 *aciphylla* were suspended in DMSO to obtain an original concentration of 150 mg/mL.

274 Cells were subcultured in 96-well plates with 3500 cells/well in 100  $\mu$ L of growth medium  
275 (Dulbecco's Modified Eagle Medium, DMEM) supplemented with 10% foetal bovine serum  
276 50 U/mL penicillin and 50  $\mu$ g/mL streptomycin). After 24 h of incubation (in humidified  
277 atmosphere, 5% CO<sub>2</sub> at 37 °C), the medium was discarded and replaced with 5 dilutions of  
278 the plant extracts in DMEM (at 300, 150, 75, 32 and 16  $\mu$ g/mL) in quadruplicate. Cellular  
279 growth control was performed using medium with 0.2% DMSO. After an incubation period of  
280 48 h, the medium was discarded and replaced with DMEM containing 0.5 mg/mL MTT (3-  
281 (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). After 1 h 30 of incubation at  
282 37°C in the 5% CO<sub>2</sub> incubator, the water-insoluble formazan was dissolved in 100  $\mu$ L  
283 dimethyl sulfoxide and the absorbance was measured at a test wavelength of 550 nm using a  
284 UV-spectrometer (Tecan, Spark 10M). The cytotoxicity of the crude extracts or standard  
285 (camptothecin: IC<sub>50</sub>: 0.9  $\pm$  0.1  $\mu$ g/mL) was determined by comparing the absorbance of cells  
286 treated by extracts with absorbance of control cells cultured in 0.2% DMSO. Data were  
287 expressed as percentages of inhibition calculated according to the formula (% Cell viability =  
288 Abs cells with extract x 100 / Abs cells control) and analyzed by linear regression.

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## 293 2.5. Compound isolation and identification

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### 295 General

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

300

### 301 *Erythrina amazonica* alkaloids isolation

302 Air-dried and powdered bark (500 g) was extracted three times overnight with  
303 methanol (1 L) using gentle shaking at room temperature (3 x 1 h). Dried crude extract (37 g)  
304 was suspended in MeOH (400 mL) and precipitated at low temperature (12 h at 5°C). After  
305 centrifugation, solid phase (9.8 g without alkaloids) was put aside and supernatant (30.2 g,  
306 containing alkaloids) was evaporated and chromatographed on silica gel column eluted with  
307 successive eluent Tol/EtOAc then EtOAc /MeOH gradients. Fractionations were monitored  
308 by TLC with visualization under UV (254 and 365 nm) and revealed with Dragendorff  
309 reagent, leading to 6 fractions (A to F). Fraction D (1.1 g) was separated by centrifugal  
310 partition chromatography: CPC with a tertiary system (EtOAc/MeOH/H<sub>2</sub>O; 1:1:0.3; v/v/v) in  
311 ascending mode (8 mL/min during 30 min), then extrusion with stationary phase was realised  
312 (30 mL/min during 10 min). CPC was performed using a 250 mL rotor (SCPC-250-L, Armen  
313 Instrument) and a Shimadzu Prominence LC-20AP binary pump. Fractions were collected  
314 every minute and 10 subfractions (1 to 10) were then pooled according to TLC analysis.  
315 Fraction number 9 (providing by extrusion) was purified on preparative silica TLC (eluted by  
316 Hex/MeOH; 45:55; V/V) to obtain 9 mg of compound **1**.

317 Erysoitrine **1**: yellow amorphous solid (9 mg), identified by NMR and MS spectra (See  
318 supplementary data). HRESIMS:  $m/z$  [M+H]<sup>+</sup> 314.1762 (calcd for C<sub>19</sub>H<sub>24</sub>NO<sub>3</sub>, 314.1751).  
319 Spectral data were consistent with those of Sarragiotto (Sarragiotto et al., 1981).

320

### 321 *Myrciaria dubia* methanolic extracts from leave, seed and peel

322 600 g of leaves were extracted according previous process (used for *E. amazonica*) to  
323 obtain 65 g of dried powder. Then, crude extract was subjected to column chromatography on  
324 silica gel and sequentially eluted with *n*-hexane, ethyl-acetate and methanol. Fractionation  
325 was monitored by TLC (with visualization under UV at 254 and 365 nm, and revelation with  
326 Liebermann Burchard or anisaldehyde sulphuric acid reagent) to afford 13 fractions (F1-F13).  
327 Fractions F6 and F8 were further purified by silica gel medium-pressure column  
328 chromatography and preparative TLC using mixtures of *n*-Hex/EtOAc/MeOH of increasing  
329 polarity, leading to the isolation and identification by NMR spectroscopy of  $\beta$ -sitosterol (13  
330 mg) and betulinic acid (11 mg).

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

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

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

341

### 342 3. Results

343

#### 344 3.1. Ethnobotanical study

345

##### 346 Ethnomedical context

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

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

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

370 The results of this study are summarized in Table 1.

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376 **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: <i>sm</i> : smell, <i>ta</i> : strong taste, <i>st=sm+ta</i> , and $\emptyset$ )
<i>Abelmoschus moschatus</i> 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: <i>st</i> ) Decoction of entire fruit used to wash intimate areas. (Fr: $\emptyset$ ).
<i>Abuta grandifolia</i> (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, <i>ta</i> ).
<i>Alchornea castaneifolia</i> (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. ( $\emptyset$ ).
<i>Aristolochia iquitensis</i> O.C. Schmidt. (Aristolochiaceae), 40708	Lengua de perro	AP	Crushed and directly applied on skin infections. ( <i>sm</i> ).
<i>Artocarpus altilis</i> (Parkinson) Fosberg. (Moraceae), 35784	Pan del arbol	B, Re	Decoction of bark applied on skin infections and resin ointment for bone fractures or hernia. ( <i>ta</i> ).
<i>Aspidosperma excelsum</i> Benth. (Apocynaceae), 37478	Remocaspi	B	Decoction of bark drunk against malaria mixed with <i>C. odorata</i> for intestinal infections or external use against herpes. ( <i>st</i> ).
<i>Brosimum acutifolium</i> Huber. (Moraceae), 39422	Murure, tamamuri	B	Alcoholic maceration ("x raices"). ( <i>sm</i> ).
<i>Calycophyllum spruceanum</i> (Benth.) Hook. (Rubiaceae), 28182	Capirona	B	Decoction of bark applied on pimples and cold sores, also in mixture APCT*. ( <i>sm</i> ).
<i>Campsiandra angustifolia</i> Spruce ex Benth. (Fabaceae), 36287	Huacapurana	B	Alcoholic maceration ("x raices"). ( <i>sm</i> ).
<i>Capsicum annuum</i> L. (Solanaceae), 37872	Macusari	Fr+S	Decoction mixed with tobacco juice against skin inflammations, or mixed with <i>C. trinitatis</i> for shamanic use against sorcery aggression. ( <i>ta</i> ).
<i>Cariniana decandra</i> Ducke. (Lecythidaceae), 28022	Tahuari	B	Alcoholic maceration as "x raices", for general purification or skin infections. ( <i>sm</i> ).
<i>Cecropia ficifolia</i> Warb. ex Sneath. (Urticaceae), 39552	Cetico	L	Juice of crushed young leaves applied against conjunctivitis. ( $\emptyset$ ).
<i>Cedrela odorata</i> L. (Meliaceae), 033958	Cedro rojo	B	Bark decoction mixed with <i>A. excelsum</i> against malaria and intestinal infections. ( <i>st</i> ).
<i>Ceiba pentandra</i> (L.) Gaertn. (Malvaceae), 33496	Lupuna blanca	B	Shamanic use in sorcery with reputation of symbolic poison. ( <i>sm</i> ).
<i>Chenopodium ambrosioides</i> L. (Amaranthaceae), 32480	Paico	AP	Decoction with <i>S. mombin</i> , <i>Malachra ruderalis</i> , <i>Plantago major</i> , <i>H. crepitans</i> with salt against sores and epidermal mycosis (external use). Crushed leaves ingested against intestinal parasites. ( <i>sm</i> ).
<i>Cissus ulmifolia</i> (Baker) Planch. (Vitaceae), 39079	Sapohuasca	AP	Alcoholic or aqueous maceration of leaves drunk against hernia or intestinal disorder. ( <i>sm</i> ).
<i>Clusia amazonica</i> 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. ( <i>ta</i> ).
<i>Copaifera paupera</i> (Herzog) Dwyer (Fabaceae), 33420	Copaiba	Re	2 drops in hot water with <i>Uncaria tomentosa</i> drunk against gastritis, or mixed with <i>J. curcas</i> against pulmonary infections. External application against venereal diseases. ( <i>ta</i> ).
<i>Cornutia odorata</i> (Poepp.) Poepp. ex Schau (Lamiaceae), 21637	Sacha mukura	AP	Decoction drunk against respiratory infections with fever. ( <i>st</i> ).
<i>Couroupita guianensis</i> 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. ( <i>st</i> ).
<i>Crescentia cujete</i> L. (Bignoniaceae), 33852	Huingo	Fr	Decoction with <i>G. americana</i> and termites nest ( <i>Isoptera</i> spp), concentrated and drunk against pulmonary infections. Decoction with <i>A. excelsum</i> against malaria. ( <i>ta</i> ).
<i>Croton trinitatis</i> Millsp. (Euphorbiaceae), 40990	Catahuio macho	L	Decoction of leaves or burnt stems applied on infected sores. ( <i>sm</i> ).
<i>Dracontium spruceanum</i> (Schott) G.H.Zhu (Araceae), 38014	Jergon sachá	T	Pulverized dried tuber applied on sore or snake bit. ( <i>sm</i> ).
<i>Eleutherine bulbosa</i> (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. ( <i>st</i> ).
<i>Erythrina amazonica</i> Krukoff. (Fabaceae), 037513	Amasisa	B	Decoction of bark used to wash skin infections (dermatitis, herpes, vaginitis ...) or mixed in APCT*. Alcoholic maceration with <i>M. alliacea</i> as external or oral administrations. ( <i>sm</i> ).
<i>Euterpe precatoria</i> Mart. (Arecaceae), 26315	Huasaí	R	Decoction drunk as depurative and against malaria. ( $\emptyset$ ).
<i>Ficus insipida</i> 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 <i>S. mombin</i> ). ( <i>ta</i> ).
<i>Genipa americana</i> L. (Rubiaceae), 33811	Huito	Fr, Re	Decoction with <i>C. cujete</i> and termites nest ( <i>Isoptera</i> spp), concentrated and drunk against pulmonary infections. ( <i>ta</i> ).
<i>Grias neuberthii</i> 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: <i>st</i> , S: <i>ta</i> ). Decoction of bark drunk against malaria. (B: <i>st</i> ).
<i>Hura crepitans</i> L. (Euphorbiaceae), 35787	Catahua	B, Re	Mixed in decoction (cf. <i>C. ambrosioides</i> ), or applied as plaster with <i>M. esculenta</i> against venereal diseases. Shamanic use in sorcery (caustic resin that must be used diluted). ( <i>st</i> ).
<i>Hymenaea oblongifolia</i> Huber. (Fabaceae), 39395	Azucar huaio	B	Alcoholic maceration ("x raices"). ( <i>sm</i> ).

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

<i>Sceliphron</i> sp. (Sphecidae)	Nido de avispa	Nest	Alcoholic concentrated maceration mixed with camphor applied against mumps. Aqueous maceration with <i>Malachra ruderalis</i> ("malva") and salt to obtain a plaster against skin inflammation and mycosis. (Ø).
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377 AP: aerial parts, B: stem bark, BFr: bark of fruit, Br: branch, Fr: fruit, L: leaves, R: root, Re: resin, T: tuber,  
 378 APCT\*: mixture of Amasisa, Papailla, Capirona and/or Tangarana (disinfectant); "x raices": mixture of barks  
 379 usually prepared as a tonic alcoholic maceration. Organoleptic properties: *sm*: smell, *ta*: strong taste, *st=sm+ta*,  
 380 and Ø without salient taste or flavor. Plant species that have not been tested in this study (reported in the table  
 381 with their entire scientific names) were previously evaluated with similar conditions (Roumy et al., 2015).  
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384 Additional information on several species and preparation from Table 1 are reported here:

385 - "x raices": called "7 or 21 raices" ("raices" meaning "roots") are mixtures of barks (genuine  
 386 preparations were constituted of roots, but now, they are widely prepared with barks which  
 387 are easier to collect). Those preparations are usually used as "tonic", to reinforce bodily  
 388 functions and libido or against rheumatism.

389 - The beverage prepared with *Manihot esculenta* called *masato* was obtained by fermentation  
 390 of the crushed tuber in water in presence of *Saccharomyces cerevisiae* var. *bayanus* (1%)  
 391 during 3 and 7 days at 30 °C in laboratory, then filtered extracts were dried and tested *in*  
 392 *vitro*.

393 - Adulterations were observed for some species: *tunchi albahaca* (*Lantana trifolia*) sold as  
 394 *catahuio embra* for replacement under the same vernacular name because the original  
 395 species could not be provided at this time. It has also been reported that some vernacular  
 396 names, such as *sapohuasca* and *amasisa* could refer to different species (*Cissus ulmifolia* or  
 397 *C. sicyoides* and *Erythrina amazonica* or *E. fusca*).

398 - Some specific traditional uses could not be evaluated with "*in vitro*" experimental conditions  
 399 e.g.:

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

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

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

411 "Baño de florecimiento": means "bath for symbolic blossoming", administered to heal  
 412 or purify the body from misfortune or injury (called "saladera"), it is a superficial bath or  
 413 an exposure to the vapor of a plant decoction (e.g. use of *Xanthosoma violaceum* with  
 414 *Hura crepitans* and *Jatropha gossypifolia*). These rituals of "washing" or "purifying" the  
 415 body had a symbolic efficacy within their cultural context, but these plants were not  
 416 really employed as disinfectant agents even if some of them contained a little bit of  
 417 antibacterial essential oil as *J. gossypifolia* leaves (e.o.: 0.03 - 0.32 %; Reis et al., 2013;  
 418 Sunday et al., 2016).

419 - Quechua language and culture have an important influence on plant taxonomies and usually  
 420 refer to the organoleptic properties of vegetal species, such as color, shape and odor. For

421 instance, *Ayahuma* means “black head” and refers to the “dark” properties of this round fruit  
422 in sorcery (*Couroupita guianensis*); *Mishu isma*, means “cat’s stool” and refers to the musky  
423 smell of *Abelmoschus moschatus* seeds. The same influence of organoleptic characteristic is  
424 observed for Spanish denominations: e.g. *Ubos colorado* for “colorful grape” from *Spondias*  
425 *mombin* tree. These examples illustrated the important influence of the organoleptic  
426 properties on plant identifications or uses as previously described (Geck, et al, 2017;  
427 Bennett, 2007), as well as their mnemonic functions: “Plants that are both empirically  
428 effective and easy to remember are more likely to be retained in oral traditions” (Shepard,  
429 2004).

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431

### 432 3.2. Results of biological tests

433

434 Results constituted, with similar previous data (Roumy et al, 2015), a contribution to  
435 the “materia medica for infectious diseases in the Amazonian district of Loreto” (Heinrich,  
436 2018). The evaluation of antimicrobial activities of the 59 plant species (33 families, 72  
437 simple extracts, 7 mixtures and 2 fermented preparations), and one insect nest extract  
438 (*Sceliphron* sp.) showed antimicrobial activities ( $MIC \leq 0.15$  mg/mL) for 14 plant extracts  
439 among 72 ( $14/72 = 19.4\%$ ), mainly composed of barks ( $8/14 = 57.1\%$ ).

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

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

454 The three plant species selected for chemical study, *Erythrina amazonica*, *Myrciaria dubia*  
455 and *Ocotea aciphylla*, were also characterized by a low cytotoxicity in HepG2 cells ( $IC_{50} >$   
456  $0.3$  mg/mL), demonstrating a specific antibacterial activity.

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## 459 4. Discussion

460

### 461 4.1. Antimicrobial activities and organoleptic drugs properties

462

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

465 had antimicrobial activity with  $MIC \leq 0.3 \mu\text{g/mL}$ . These results may be correlated to a higher  
466 content of tannin or a best stability of essential oil in these rough drugs, explaining the  
467 antimicrobial activity and the sensory cues (bitter or fragrant). These observations may be  
468 enlightened by 2 different ways:

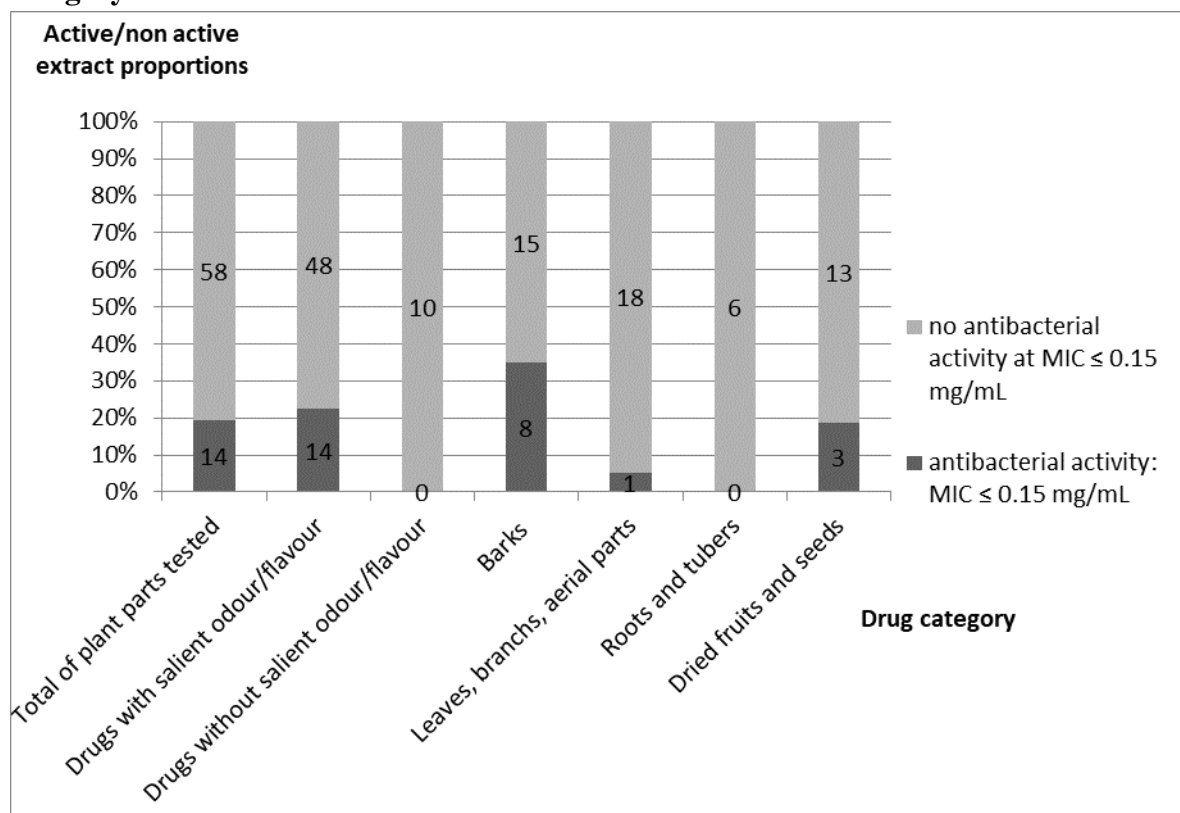
469 First of all, in the Amazonian pharmacopeias, organoleptic properties are excellent  
470 guides for selecting or memorizing medicinal plants (Leonti et al., 2001) and plants that are  
471 both empirically effective and easy to remember are more likely to be retained in oral  
472 traditions (Shepard,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

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

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

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489 **Fig. 1. Distribution of antimicrobial activity by drug**  
 490 **category**



491  
 492 *The trend observed for antimicrobial activity in case of bark with chemosensory cues were statistically*  
 493 *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*  
 494 *mg/mL (p = 0.26 and p = 0.19)*

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496

#### 497 4.2. Antimicrobial plant spectra and phytochemicals

498

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



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

516 A search in the literature indicated that the potential antibacterial agents of some of the  
517 investigated plants had already been characterized. Among the 12 species characterised by a  
518 relevant antibacterial activity ( $MIC \leq 0.15$  mg/mL), 8 bark extracts were brought out:  
519 *Aspidosperma excelsum* (with indole monoterpene alkaloids as antimicrobial compounds:  
520 Correia et al., 2008), *Brosimum acutifolium* (flavonoids and lignoids as active compounds,  
521 *ibid.*), *Erythrina amazonica* (flavonoids, pterocarpan and erythrinan alkaloids as antibacterial  
522 compounds identified in the genus *Erythrina*: Wanjala et al., 2002, Yenesew et al., 2005, De  
523 Avila et al., 2018), *Hura crepitans* (alkaloids, flavonoids and tannins: Oloyed and Olatinwo,  
524 2014), *Ocotea aciphylla* (neolignans: Felicio, et al., 1986; gallic tannins and aromatic  
525 compounds revealed in this study), *Spondias mombin* (tannins, flavonoids, anthraquinones  
526 and saponins: Ayoka et al., 2008), *Swartzia polyphylla* (flavonoids, pterocarpan and saponins:  
527 Osawa et al., 1992), *Vismia macrophylla* (bark or leaves composed of essential oil with  $\gamma$ -  
528 bisabolene and cytotoxic anthrone derivatives, Buitrago et al., 2015; Hussein et al., 2003).  
529 Relevant activity was also observed for *Copaifera paupera* (resin: diterpenes then lipids and  
530 tannins; Arruda et al., 2019; Correia et al., 2008), *Persea americana* (seed containing  
531 antibacterial phenolic compounds and lipids: Sidrim et al., 2009), *Myrciaria dubia* (seed or  
532 fruit bark with tannin and phloroglucinol as antibacterial compounds, Myoda et al. 2010;  
533 Kaneshima et al., 2017) and *Virola pavanis* (root, neolignans and other phenylpropanoids  
534 derivatives: Ferri and Barata, 1992; Zacchino et al., 1998). Bibliographic research on barks  
535 species from *Erythrina amazonica* and *Ocotea aciphylla* or leaves from *Myrciaria dubia* did  
536 not allow identification of their antimicrobial content, justifying here their chemical study  
537 (Ueda et al., 2004).

538

#### 539 4.3. Activity-guided isolation of antimicrobial compounds

540

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

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

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

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

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

566

## 567 **5. Conclusion**

568

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

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

579

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

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

593

## 594 **Conflict of interest**

595 The authors declare that they have no conflict of interest.

596

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598  
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605

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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		Enterobacter aerogenes		Enterobacter cloacae			Klebsiella pneumoniae		Serratia marcescens		Providencia stuartii		Salmonella sp.		Yersinia pseudotuberculosis		Acinetobacter baumannii		Pseudomonas aeruginosa		Stenotrophomonas 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	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	<i>Spondias mombin</i>	B	1.2	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	0.6	-	-	-	-	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	<i>Aspidosperma excelsum</i>	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	0.6	0.6	0.6	0.3	1.2	0.3	1.2											
Asteraceae	<i>Tessaria integrifolia</i>	Br	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	0.6	-	-	-	-	-	-	-	-	-	-	-	-											
Celastraceae	<i>Maytenus macrocarpa</i>	B	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-	-	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	1.2	-										
Clusiaceae	<i>Clusia amazonica</i>	B + R	-	-	-	-	-	-	-	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	0.6	0.6	0.6	0.6	0.6	1.2											
Cucurbitaceae	<i>Momordica charantia</i>	AP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1.2	-	-	1.2	1.2	0.6	1.2	0.6	-	0.6	0.6	0.3	1.2	-	-											
Cyperaceae	<i>Scleria macrophylla</i>	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-												
Euphorbiaceae	<i>Alchornea castaneifolia</i>	L	-	-	-	-	-	-	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	0.6	-	-	1.2	0.6	1.2	-	0.6	1.2	-	-	-	-	-	-	1.2	-										
	<i>Croton trinitatis</i>	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	1.2	1.2	1.2	1.2	1.2	-										
	<i>Hura crepitans</i>	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	0.3	0.6									
	<i>Jatropha curcas</i>	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	1.2	1.2	-										
Fabaceae	<i>Campsiandra angustifolia</i>	B	-	-	-	-	-	-	-	0.6	-	-	-	-	-	-	0.6	-	-	1.2	0.6	0.6	0.6	1.2	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	0.6	0.6	1.2										
	<i>Copaifera paupera</i>	Re	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	1.2	0.6	-	0.6	0.3	0.15	0.3	-	-	-	-	-												
	<i>Erythrina amazonica</i>	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	0.3	0.3	0.3	0.15	0.3	0.15	0.3	0.15	0.3	0.15	0.3	0.15	0.15	0.3	0.3	-												
	<i>Hymenaea oblongifolia</i>	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	0.6	-											
	<i>Lonchocarpus nicou</i>	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-												
	<i>Swartzia polyphylla</i>	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	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.6										
<i>Zygia latifolia</i>	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	-	-	-	1.2	1.2	-	1.2	-	1.2	0.6	1.2	0.6	0.3	0.6	1.2	1.2	1.2	0.6	0.6	0.6	0.6	0.6	1.2											
Hypericaceae	<i>Vismia macrophylla</i>	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	1.2	0.15	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	<i>Eleutherine bulbosa</i>	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	1.2	-	-	0.6	0.6	0.3	0.6	0.6	0.3	0.6	0.3	0.6	0.3	0.6	1.2	0.6	1.2										

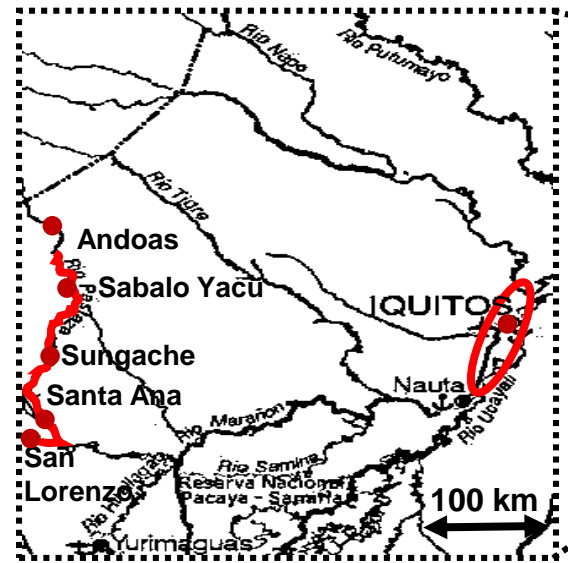




AP: aerial parts. B: stem bark and branch. EP: entire plant. Fl: flower. Fr: fruit. L: leaves. R: root. Re: resin. T: tuber, BFr: bark of fruit. MIC (mg/L) of positive controls: nt: not tested na: not active at 64 mg/L. Gentamicin. S:  $\leq 4$ . R:  $> 8$ ; Vancomycin. S:  $\leq 4$ . R:  $> 16$ ; Amoxicillin.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

Color code for extracts with MIC  $< 0.15$  mg/mL

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



**Ethnopharmacological survey**



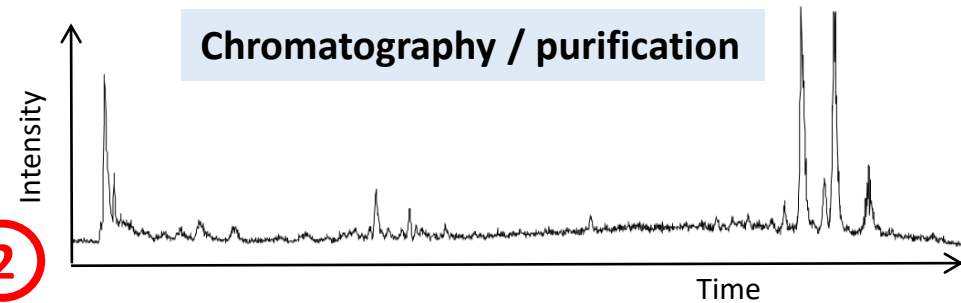
1

**Plant extraction**

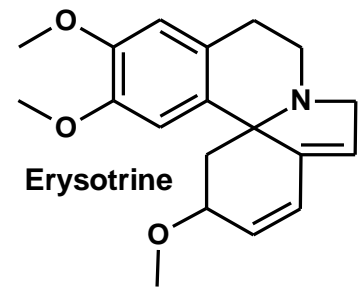


2

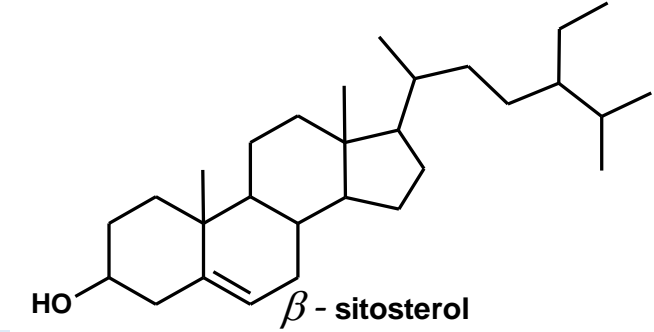
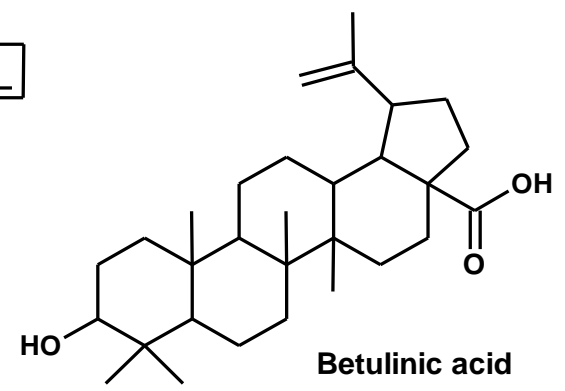
**Chromatography / purification**



**Isolation and identification of compounds**



3



**Antimicrobial tests**

