Journal of Ethnopharmacology 280 (2021) 114473

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Pharmacological validation of *Solanum mammosum* L. as an anti-infective agent: Role of solamargine

Billy Cabanillas [a,b], François Chassagne [c], Pedro Vásquez-Ocmín [c], Ali Tahrioui [d], Sylvie Chevalier [d], Marieke Vansteelandt [c], Asih Triastuti [e], Carlos A. Amasifuen Guerra [f], Nicolas Fabre [c], Mohamed Haddad [c,*]

[a] Laboratorios de Investigación y Desarrollo, Universidad Peruana Cayetano Heredia (UPCH), Lima, 34, Peru
[b] Instituto de Investigaciones de la Amazonía Peruana, Avenida Abelardo Quiñonez Km. 4.5, Iquitos, Peru
[c] UMR 152 PharmaDev, Université de Toulouse, IRD, UPS, France
[d] Laboratoire de Microbiologie Signaux et Microenvironnement, LMSM EA4312, Normandie Université, Université de Rouen Normandie, Évreux, France
[e] Department of Pharmacy, Universitas Islam Indonesia, Yogyakarta, 55584, Indonesia
[f] Instituto Nacional de Innovación Agraria, Dirección de Recursos Genéticos y Biotecnología, Avenida La Molina 1981, La Molina, Lima, 15024, Peru

ARTICLE INFO

Keywords:
Solanum mammosum
Candida albicans
Trichophyton mentagrophytes
Pseudomonas aeruginosa
Bioautography
Solamargine
LC-HRMS

ABSTRACT

Ethnopharmacological relevance: Fungal and bacterial infections remain a major problem worldwide, requiring the development of effective therapeutic strategies. *Solanum mammosum* L. (Solanaceae) ("teta de vaca") is used in traditional medicine in Peru to treat fungal infections and respiratory disorders *via* topical application. However, the mechanism of action remains unknown, particularly in light of its chemical composition.

Materials and methods: The antifungal activity of TDV was determined against *Trichophyton mentagrophytes* and *Candida albicans* using bioautography-TLC-HRMS to rapidly identify the active compounds. Then, the minimum inhibitory concentration (MIC) of the fruit crude extract and the active compound was determined to precisely evaluate the antifungal activity. Additionally, the effects of the most active compound on the formation of *Pseudomonas aeruginosa* biofilms and pyocyanin production were evaluated. Finally, a LC-HRMS profile and a molecular network of TDV extract were created to characterize the metabolites in the fruits' ethanolic extract.

Results: Bioautography-TLC-HRMS followed by isolation and confirmation of the structure of the active compound by 1D and 2D NMR allowed the identification solamargine as the main compound responsible for the anti-*Trichophyton mentagrophytes* (MIC = 64 μg mL^{-1}) and anti-*Candida albicans* (MIC = 64 μg mL^{-1}) activities. In addition, solamargine led to a significant reduction of about 20% of the *Pseudomonas aeruginosa* biofilm formation. This effect was observed at a very low concentration (1.6 μg mL^{-1}) and remained fairly consistent regardless of the concentration. In addition, solamargine reduced pyocyanin production by about 20% at concentrations of 12.5 and 50 μg mL^{-1}. Furthermore, the LC-HRMS profiling of TDV allowed us to annotate seven known compounds that were analyzed through a molecular network.

Conclusions: Solamargine has been shown to be the most active compound against *T. mentoagrophytes* and *C. albicans in vitro*. In addition, our data show that this compound affects significantly *P. aeruginosa* pyocyanin production and biofilm formation in our conditions. Altogether, these results might explain the traditional use of *S. mammosum* fruits to treat a variety of fungal infections and respiratory disorders.

1. Introduction

Solanum mammosum L. (Solanaceae) is an annual or tender perennial plant, reputed to be a tropical American plant, considered native to Central and South America (Acevedo-Rodríguez and Strong, 2008), named in the traditional medicine of Peruvian Amazon "*teta de vaca*" (TDV).

S. mammosum was introduced to the Old World tropics for ornamental use as well as medicinal and food purposes (Lim, 2013) and reported as invasive in Cuba, Philippines, Fiji, Tonga, and Hawaii (Oviedo et al., 2012; Space and Flynn, 2002; Pier, 2014). The fruit, decorative, is a toxic piriform berry, initially light green, to a bright yellow color

* Corresponding author.
E-mail address: mohamed.haddad@ird.fr (M. Haddad).

https://doi.org/10.1016/j.jep.2021.114473
Received 21 May 2021; Received in revised form 26 July 2021; Accepted 27 July 2021
Available online 31 July 2021

when, tending over time to orange-yellow with white spongy mesocarp and numerous semi lenticular seeds. *S. mammosum* has traditionally been used to treat athlete's foot among hunter groups in Peru (Jovel et al., 1996; Polesna et al., 2011), Belize (Arnason et al., 1980) and Trinidad (Lans et al., 2001), by rubbing leaf juice or cut fruit onto afflicted areas. In Bolivia, the fruits are mashed and rubbed over the affected area to treat skin ulcer, scabies, furunculosis and rashes (Muñoz et al., 2000; Hajdu and Hohmann, 2012). In Guatemala and in the Philippines, leaves, fruits and seeds are also used in the treatment of respiratory disorders such as asthma, cough, cold and sinusitis (Caceres et al., 1991). Other studies have reported various pharmacological properties of the plant including antioxidant, anticancer, antimalarial and molluscicidal activities (Burkill, 1966; Grieve, 1971; Muñoz et al., 2000; Wiart et al., 2004; DeFilipps et al., 2004; Chaiyasit et al., 2006; Stuart, 2010; Crommett, 2011). Additionally, due to its toxicity, it has been used in the past as an insecticide, rat poison (Flores, 1984) and for fish catching (Levin et al., 2005). Litterature data reports the presence of many potentially toxic substances, including alkaloids and saponins that give the plant its pharmacological properties. *S. mammosum* fruit was found to contain solasodine along with other steroidal glycoalkaloids, including solasonine, solamargine and β-solamarginine (Tarigan, 1980), as well as diosgenin and phytosterols (Sawariam, 1986). As *S. mammosum* fruits and/or leaf juices are rubbed onto afflicted areas to treat various health ailments related to the possible presence of *T. mentagrophytes* (athlete's foot) and *C. albicans*, we designed a study to identify the major antifungal compounds in *S. mammosum* fruit using bioautography-TLC-HRMS, a combination of cutting-edge microbiological, chromatographic, and spectrometric tools. Additionally, we evaluated the main active compound of the fruit extract (*i.e.*, solamargine) for the first time on *P. aeruginosa* pyocanin production and biofilm formation, since *Solanum mammosum* is used in the treatment of respiratory disease due to its antimicrobial activity (Caceres et al., 1991). Finally, a dereplication and molecular network analysis strategy was used to explore the chemical composition of *S. mammosum* fruit through UHPLC-ESI-MS/MS.

2. Material and methods

2.1. Plant material

S. mammosum fruits were collected in the Allpahuayo Mishana National Reserve, Iquitos, Maynas Province (Peru) (3°58′02.3″S 73°25′03.9″W; −3.967295, −73.417754) by Billy Cabanillas and Mohamed Haddad. Plant identification was performed by the botanist C. A. Amasifuen Guerra (Voucher N° 26646, Museum of Natural History of Lima, Peru). Authorization to collect the plant was obtained from relevant authorities (authorization SERNANP N° 010-2017-SERNANP-RNAM/JRR).

2.2. Drug and chemicals

Methanol, ethanol, chloroform and all solvents used for extraction, TLC development and LC/MS grade solvents as well as MHA, glucose anhydrous, SDA, and LB were purchased from Fisher Chemicals (Leicestershire, UK). MTT and DMSO were obtained from Sigma-Aldrich (Saint-Louis, MO, USA). Water was deionized using Milli-Q water purification system (Millipore, Bedford, MA, USA). PBS Gibco® was obtained from Thermo Fisher Scientific (Waltham, MA, USA). RPMI 1640 was purchased from Corning (Corning, NY, USA).

2.3. Preparation of extracts, sample preparation and stock solutions for bioautography

The pulp of 20 fresh fruits (~150 g) was extracted by maceration in MeOH (1 L) at room temperature for 24 h using an orbital shaker from Thermo Scientific (Waltham, MA, USA). After filtration and evaporation to dryness under reduced pressure (Rotavapor R-100 (Buchi, Flawil, Switzerland), the concentrated residual extracts (21.6 g) was stored at -20 °C in a dry airtight container until further use. A stock solution of *S. mammosum* in MeOH (10 mg mL^{-1}) was prepared, aliquoted in different vials (2 mL), then stored at - 80 °C for further analysis. Individual stock solutions of amphotericin B (0.5 mg mL^{-1}, Sigma) (200 µL aliquot, positive control) were prepared in DMSO (analytical grade, Sigma) and used as a positive control by spotting onto the plate 1 µL of the stock solution with disposable micropipettes.

2.4. Fungal and bacterial strains, media, and growth conditions

Trichophyton mentagrophytes (18748) and *Candida albicans* strains (10231, 90028) were purchased from ATCC. The fungi were subcultured and routinely maintained on SDA at 4 °C in a cold room until use. A sterile swap was used to inoculate the fungi into the liquid medium (MHA).

Pseudomonas aeruginosa H103 is a derivative of *P. aeruginosa* wild-type PAO1 strain (Hancock and Carey, 1979). Planktonic cultures were grown aerobically for 24 h at 37 °C in LB broth on a rotary shaker (180 rpm) from an initial inoculum adjusted to an absorbance at 580 nm of 0.08.

2.5. Inoculum for the bioautography assay

Inoculum is prepared by picking five distinct colonies of approximately 1 mm from 24 h old culture grown on SDA and incubated at 25 ± 2 °C for *T. mentagrophytes* and 35 ± 2 °C for the *Candida albicans* strains. Colonies were suspended in 5 mL of sterile 0.85% saline solution and the turbidity of the resulting suspension was adjusted to yield 1×10^6–5×10^6 cells mL^{-1} (*i.e.* 0.5 McFarland standard). MHA was used as the solid media for the *T. mentagrophytes* and *C. albicans* overlays. The molten media were maintained in a water bath at 45 °C and inoculated with the inoculum. The final concentration in the solid medium was approx. 10^5 cells mL^{-1}. The suspension was prepared immediately before carrying out the test.

2.6. Thin-layer chromatography (TLC)

TLC were carried out on a precoated silica gel 60 F254 (Merck, Darmstadt, Germany), with an appropriate solvent system in a classical TLC chamber. One µL of the amphotericin B stock solution and 5 µL of *S. mammosum* extract at the concentrations of 10, 20 and 30 mg. mL^{-1}were spotted on TLC plates. Standard mobile phases $CHCl_3$/MeOH/H_2O (65:40:10, v/v/v) and $CHCl_3$/MeOH (50:50, v/v) were used to separate components over a wide range of polarities. The standard solutions/extract samples were applied to the TLC plate by using an automatic TLC sampler ATS4 (CAMAG, Muttenz, Switzerland) with the following application conditions: filling speed: 15 µL s^{-1}, pre-dosage volume: 200 nL, dosage application speed: 150 nL/s, rinsing cycle 1 with methanol/water (9:1, v/v), rinsing vacuum time: 4 s, filling vacuum time: 1 s. The TLC plates were prepared in triplicate: after examination of all developed chromatograms under ultra-violet light at 254 and 366 nm (CAMAG Universal UV lamp TL 600), one plate was sprayed with vanillin–sulphuric acid reagent, while others were kept for bioautography and TLC-MS, respectively.

2.7. Bioautography: agar-overlay method

Bioautographic agar overlay method was used following the protocol described in Rahalison et al. (1991) with slight modifications. Briefly, TLC plate was placed in a Petri dish and covered with approximately 10 mL of a thin layer of MHA inoculated with *T. mentagrophytes* or *C. albicans*. After solidification of the medium, TLC plates were incubated overnight at 25 ± 2 °C or 35 ± 2 °C in polyethylene boxes lined with moist chromatography paper. The bioautograms were sprayed with

an aqueous solution (2.5 mg mL^{-1}) of MTT and incubated for 4 h at 30 °C. Dehydrogenases of living microorganisms convert these salts into colored formazans, and as a result yellow zones of inhibition are observed on a purple background. Then, the bioautography assay was analyzed by observing inhibition zones (no color) and non-inhibition zone (purple color). Amphotericin B was used as a reference compound for the determination of inhibition zone.

2.8. Characterization of bioactive compounds using mass spectrometry

A TLC-MS Interface with an oval extraction head of 4 × 2 mm (CAMAG, Muttenz, Switzerland) was connected between an UHPLC (Dionex UltiMate3000, Dionex, USA) and an Orbitrap mass spectrometer (LTQ XL, 21880, Thermo Fisher Scientific, USA). The bioactive zones on the TLC plate were marked with a soft pencil based on their R_F value and extraction was performed with a mixture of methanol and water (95:5, v/v) at a flow rate of 0.5 mL/min provided by the UHPLC pumps. Mass spectrometric analysis was carried out in negative- and positive-ion modes. ESI parameters were set as follows: heater temperature 300.0 °C, capillary temperature 350.0 °C, capillary voltage 10.0 V, sheath gas flow rate 10 arbitrary units, aux gas flow rate 5 arbitrary units, tube lens 80 V, Ion spray voltage 3.50 kV. Data was acquired and recorded by Thermo Xcalibur Qual Browser software. Different collision energies were applied for MS/MS analysis in order to obtain more information about fragment ions of the target constituents.

2.9. Isolation of the active compound

The methanolic extract (1 g) of the fruit of *S. mammosum* was subjected to a flash chromatography instrument (Spot Ultimate, Armen, France) on a silica column (Chromabond® Flash RS 25 SiOH 40–63 μm) eluted with the solvent systems $CHCl_3$/MeOH/H_2O (80:20:2, v/v/v) to give six main sub-fractions (B1–B6), including pure solamargine (34.7 mg).

2.10. Minimum inhibitory concentrations (MIC) assays

C. albicans strains (10231, 90028) were purchased from American Type Culture Collection (ATCC). MIC values were determined by the broth microdilution method according to the CLSI (2008). The yeast was grown at 35 °C on SDA plates for 48 h. The inoculum was prepared by suspending scraped cell mass in 0.85% NaCl solution, adjusted to 0.5 Mc Farland standard with a spectrophotometer at 530 nm, then diluted to obtain a final suspension of 5.0 × 10^2 to 2.5 × 10^3 cells per mL. RPMI 1640 medium buffered with MOPS and supplemented with dextrose was used as a growth media. In a 96-well plate, plant extracts (10 mg mL^{-1} in DMSO) were serial diluted in RPMI 1640 medium so that 8 concentrations in the range of 4–512 μg mL^{-1} were obtained. The working culture was then added to all wells, and the plates were incubated at 35 °C for 48 h. A spectrophotometer was used to determine the MIC by reading the 96-well plate at 600 nm. The MIC was defined as the lowest concentration of plant extract that completely inhibits growth of *C. albicans* in the wells as detected by the unaided eye (CLSI, 2008). Each test was performed in triplicates. Amphotericin B was used as a positive control. Cultures without plant extracts or antifungal were employed as negative control.

2.11. Pyocyanin quantification assay

Pyocyanin quantification assay was carried out as described previously by Tahrioui et al. (2020). *P. aeruginosa* H103 cells untreated and treated with solamargine were grown in a 96-well microtiter plate at 37 °C for 24 h on a rotary shaker (180 r.p.m). One volume of chloroform was used to extract free-cell supernatants samples. Then, ½ volume of 0.5 M HCl was added to the chloroform layer (blue layer). The absorbance of the HCl layer (red-pink layer) was recorded at 520 nm and the data were normalized for bacterial cell density (A580 nm).

2.12. Quantitative biofilm assay

To assess the propensity of *P. aeruginosa* H103 strain to form biofilms in the presence of solamargine, we performed crystal-violet-adhesion assays as described by O'Toole (2011). Briefly, overnight cultures were inoculated into a fresh medium and grown for 24 h in a 96-well microtiter plate. Cell growth was determined from A580 nm. Biofilm was measured by discarding the medium, rinsing the wells with water and staining any bound cells with crystal violet at 0.1%. The dye was dissolved in 30% w/v acetic acid and A595 nm was determined in each experiment, background staining was adjusted by subtracting the crystal violet bound to inoculated controls.

2.13. LCMS analytical methodology

The methanolic extract of *S. mammosum* was carried out by UHPLC-DAD-LTQ Orbitrap XL instrument (Ultimate 3000, Thermo Fisher Scientific), which was equipped with an electrospray ionization probe (ESI). Chromatographic separations were performed on an Acquity BEH C18 column (100 × 2.1 mm i.d., 1.7 μm, Waters, USA). The mobile phase comprised acidified solvents (0.1% formic acid), water (A) and acetonitrile (B) respectively. A stepwise gradient method at constant flow rate of 0.3 mL/min was used to elute the column using the following conditions: 0–0.5 min, 95% A; 0.5–12 min, 95–5% A; 12–15 min, 5% A; 15–15.5 min, 5–95% A; 15.5–19 min, 95% A. Analyses of the samples (2 μL injected) were performed by a diode array detector (DAD) from 210 to 400 nm. The column temperature was maintained at 40 °C. Mass parameter settings were: negative ESI mode, under the following conditions: capillary voltage at 3.0 kV, capillary temperature at 300 °C. Full mass spectra were recorded between 100 and 1500 Da. CID mass spectra were obtained in the data dependent mode for the four most intense ions (top 4) of each MS full scan using the following parameters: 35% normalized collision energy, isolation width 2 Da, activation Q0.250. External mass calibration was performed before starting the experiment.

2.14. Data processing

Data obtained from high resolution mass spectrometry (.raw) were first processed with MZmine 2.52 (Pluskal et al., 2010). Briefly, as a first step, the transformation of chromatograms in a peak list was realized following mass detection for the extract. Then, the chromatogram was built and deconvolutioned using ADAP chromato-builder and wavelets (ADAP) algorithms; grouping of isotope patterns (peak grouper algorithm) and a unique peaks list aligned was created. The gap filling (peak finder algorithm) in the list was also performed. Cleaning of the peaks list was carried out as follows: merge of duplicates in the list, attribution of scans MS2 to MS1 using group MS2 scans with features algorithm and finally the peak list was filtered using the peak list rows filter by keeping only the peaks with MS2 scan.

In the peaks list, the features annotations of known compounds, by looking for compounds corresponding to the molecular formula derived from HRMS data, were realized as follows: 1) identification of fragments and complexes; 2) comparison with an in-house database compiled from literature data to gather secondary metabolites isolated from *Solanum* genus and Solanaceae family (Data not shown) for the annotation of known compounds; 3) Pubchem online database was screened for annotating the other compounds. Before the exportation of the peaks list data, these latter were normalized using the linear normalizer parameters. Finally, the exportation of generated MS/MS spectra was made in MGF format and the list of mass compounds, retention times, row ID and peak heights was exported in CSV (comma-separated value). Molecular network (mass spectra similarity) was carried out using the open-source software MetGem (Olivon et al., 2018) from final MS/MS data. Values used were MS2 m/z tolerance = 0.1 Da, minimum matched peaks = 4

and minimal cosine score value = 0.65. Visualization of the network was performed on Cytoscape version 3.8.2 (Shannon et al., 2003). Compounds with the same fragmentation pathway were grouped into the same cluster.

3. Results

3.1. Bioautography-TLC-HRMS/MS, isolation and structural determination of the bioactive compound

As a preliminary assay, a bioautography-TLC-HRMS/MS of *S. mammosum* fruit extract was initially performed in order to identify the potential anti-*Trichophyton mentagrophytes* and anti-*Candida albicans* metabolites present in *S. mammosum*, as it relates to its traditional use. One main inhibition zone with R_f=0.72 was identified in both bioautographies and marked with a pencil on the duplicate TLC plate and extracted for MS analysis, using the TLC-MS interface. The workflow is shown in Fig. 1. Solamargine (**1,** Fig. 2) was identified as the main compound by TLC-HRMS (m/z = 868.5057 $[M+H]^+$; calcd for $C_{45}H_{73}NO_{15}$ 867.4980). Further confirmation was obtained by the isolation of the active spot through Combiflash® on Si60 followed by structure determination by 1D and 2D NMR and comparison with literature (Burger et al., 2018).

3.2. Minimum inhibitory concentrations assays

The results of MIC determination from TDV fruits crude extract and solamargine against *T. mentagrophytes* and two strains of *C. albicans* are shown in Table 1. TDV exhibited weak anti-*Trichophyton* and anti-*Candida albicans* activities (MIC = 256 µg mL^{-1}) when compared to Amphotericin B (100% inhibition at 8 µg mL^{-1}) whereas solamargine exhibited a moderate activity against the three strains (MIC = 64 µg mL^{-1}).

3.3. Effect of solamargine on pyocyanin production and biofilm formation

To assess the impact of solamargine on *P. aeruginosa* physiology, its effect on virulence through the pyocyanin production as well as on biofilm formation were studied. The effect of solamargine against *P. aeruginosa* was evaluated at concentrations ranging from 0.8 to 100 µg mL^{-1}, in a liquid medium using the model bacterium *P. aeruginosa* H103, a prototroph derivative of PAO1 wild-type strain. Solamargine reduced pyocyanin production by about 20% at 50 µg mL^{-1} (Fig. 3a). However, no impact was observed at low concentrations. Solamargine also led to a significant reduction of about 20% of the biofilm formation

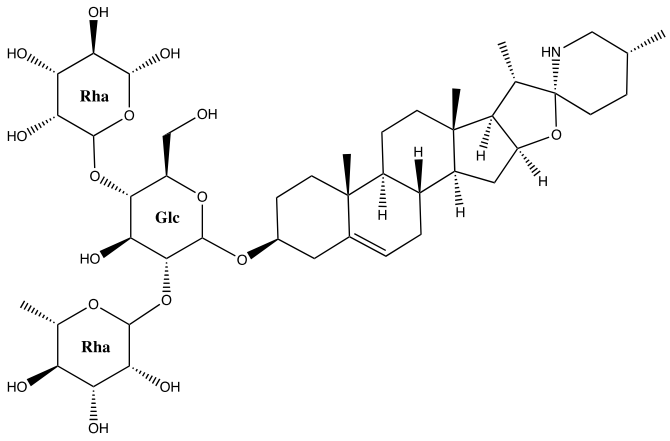

Fig. 2. Structure of solamargine **1**.

Table 1
Antifungal activity (MIC values) of solamargine on two *Candida albicans* strains and one *Trichophyton mentagrophytes* strain.

	MIC (µg.mL^{-1})		
	T. mentagrophytes	*C. albicans* 90028	*C. albicans* 10231
TDV crude fruit extract	256	256	256
Solamargine	64	64	64

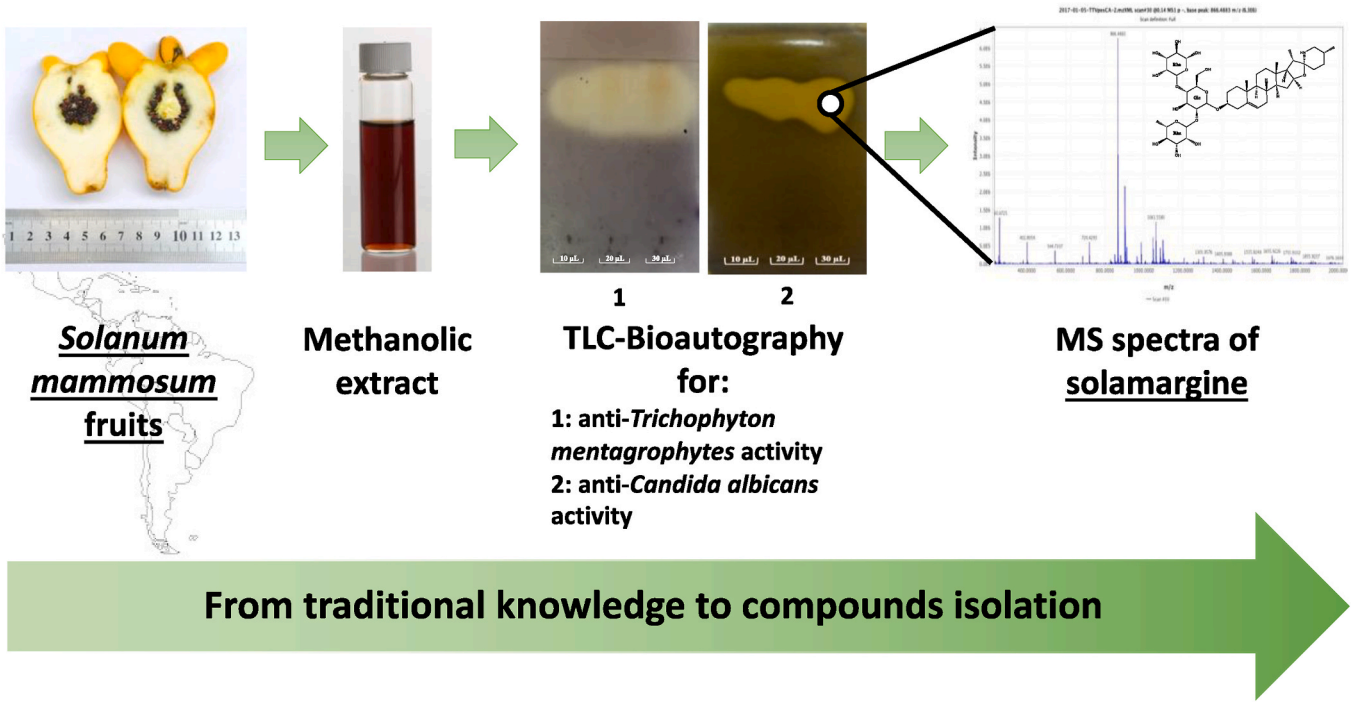

Fig. 1. Bioautography-TLC-MS workflow for anti-*Trichophyton mentagrophytes* and *anti-Candida albicans* activities.

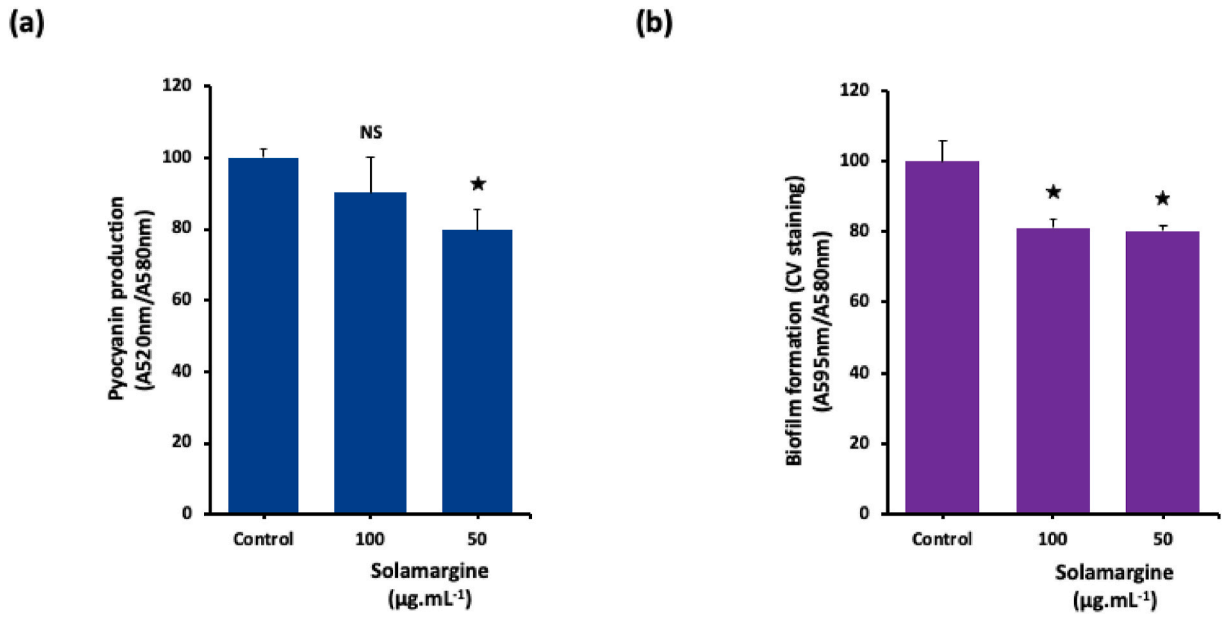

Fig. 3. Effect of solamargine on pyocyanin production and biofilm formation by *P. aeruginosa*. Statistics were achieved by a two-tailed *t*-test using Prism GraphPad. The mean with SEM were calculated and plotted. ★, $p = 0.01$ to 0.05; NS (Not Significant), $p \geq 0.05$.

(Fig. 3b). This effect was observed for a very low concentration (1.6 µg mL^{-1}) and was quite similar for the other solamargine concentrations tested (*data not shown*). Altogether, our data show that solamargine moderately affects virulence traits of *P. aeruginosa* at 50 µg mL^{-1}.

3.4. Chemical composition of TDV MeOH extract and annotation

A list of 143 peaks (Rt-*m/z*) which corresponded to the retention times and pseudomolecular ion masses of compounds were obtained from methanolic extract of *S. mammosum*, created using the MZmine software. Top priority for the annotation of the compounds in the peaks list was made for the *Solanum* genus in-house database. Thus, seven compounds were annotated (Table 2), including the active compound solamargine. Then, we crossed the information between compounds annotated and bibliographic data (activity on *T. mentagrophytes*, *C. albicans* and *P. aeruginosa*). Visualization of chemical class of compounds annotated from *S. mammosum* were placed in network according to MS/MS fragmentation similarity (Fig. 4).

4. Discussion

In this study, we investigated the anti-*Trichophyton mentagrophytes* and anti-*Candida albicans* activity of TDV MeOH extract through bioautography-TLC-HRMS and isolated the most active compound, solamargine.

S. mammosum, named in the traditional medicine of Peruvian Amazon *chucho de vaca*, *teta de vaca*, *tinta uma*, *cocona venenosa*, *tintuma*, *tinctona*, *resalgal*, *tintonilla*, *cocoán* and *chuf-cha* (Pinedo et al., 1997; Vásquez, 1997; Vega, 2001) is commonly used in Peru to treat mycosis and scabies (Roumy et al., 2007), to relieve headaches (Luziatelli et al., 2010) and as a poison to kill rats (Ayala Flores, 1984). Among the available ethnopharmacological data, several authors have reported the traditional use of different botanical parts of *S. mammosum* against fungal skin infections (Roumy et al., 2007; Hajdu and Hohmann, 2012; Polesna et al., 2011; Lim, 2013). Particularly, *S. mammosum* is specifically used to treat athlete's foot infection (Muñoz et al., 2000), a superficial inflammatory infection of the feet skin caused by dermatophyte fungi, especially *Trichophyton rubrum*, *T. mentagrophytes*, and *Epidermophyton floccosum* (Hsu and Hsu, 2012; Rinaldi, 2000). In this study, our result showed that solamargine is the main active ingredients of *S. mammosum*, exhibiting a moderate activity against *T. mentagrophytes* and *C. albicans* (MIC = 64 µg mL^{-1} against both strains), which might confirm its traditional use to treat skin fungal infections. In addition, *S. mammosum* is also traditionally used in the treatment of respiratory diseases but few studies have been done to better understand the mechanism of action. Caceres et al. (1991) carried out a screening of 68 plants used in Guatemala for the treatment of respiratory diseases. They have shown that several plants used for the treatment of respiratory infections have some *in vitro* activity against pathogenic gram-positive bacteria, including *S. mammosum* (moderate activity against *Streptococcus pneumonias*). *P. aeruginosa* is a pathogenic gram-positive bacteria responsible of repiratory infections. It is known to produce a range of virulence factors that enhance its ability to damage the host tissue. One of the most important virulence factors is pyocyanin that is highly toxic because of its redox-active and zwitterionic properties, contributing to tissue damage (Lau et al., 2004) and inducing pulmonary pathophysiology in mice (Caldwell et al., 2009). Pyocyanin is a blue redox-active secondary metabolite that is readily recovered in large quantities in sputum from patients with cystic fibrosis. Pyocyanin can cross the cell membrane and causes oxidative stress by generating reactive oxygen and nitrogen species, which allow *P. aeruginosa* to kill competitor microbes inhabiting the same niche, as well as damaging host cells or modulating their immune signaling (Morin et al., 2021). In this study, our data indicate that Solamargine has a significant effect on *P. aeruginosa* biofilm formation and pyocyanin production under our conditions, which brings new insights about the potential of this plant to treat respiratory problems.

From a chemical point of view, S. mammosum was shown to contain

Table 2
Principal molecular ions determined in the methanolic extract of S. mammosum by LCMS and corresponding compounds.

m/z [M-H]⁻	RT	Compound	Main fragments
851.2234	3.7337	Viarumacid A	689.220; 515.227; 497.196
207.0658	5.91	Ethyl caffeate	179.018; 135.162; 207.072
285.0396	4.79	Kaempferol	257.099; 199.109; 217.064
347.0976	1.5402	Tabaflavone E	161.090; 139.120; 223.017
299.0553	5.1537	Sorbifolin	284.094; 271.135; 267.188
609.1449	3.5785	Rutin	447.210; 285.054; 489.332
867.3099	5.8119	Solamargine	720.4147; 469.243; 549.159

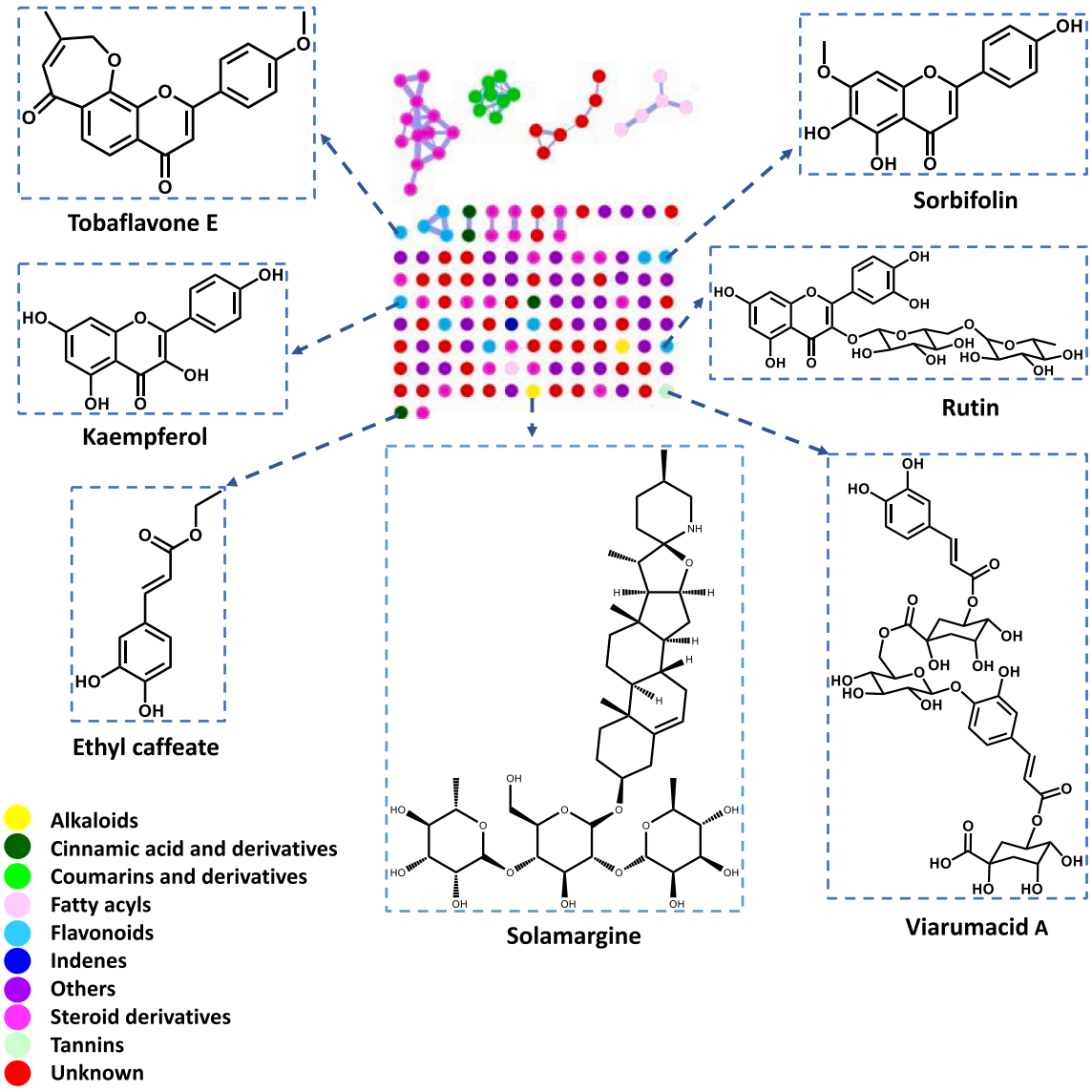

Fig. 4. Molecular networking and visualization of compounds annotated from *S. mammosum*. The compounds classification was carried out using Classy-Fire (Feunang et al., 2016).

metabolites which are both highly toxic to humans but medicinally useful. It is a source of solasodine (Telek, 1977; Roddick and Rijnenberg, 1986; Hanelt et al., 2001; Lim, 2013), a poisonous, teratogenic, alkaloidal compound that is a precursor to pharmaceutical production of contraceptive pills and has also been the subject of recent research for its diuretic, anticancer, antifungal, cardiotonic, antispermatogenetic, antiandrogenic, immunomodulatory, and antipyretic effects on the central nervous system (Patel et al., 2013). Also, solamargine is a major glycoalkaloid in *Solanum* species and especially in *S. mammosum*, which has been less studied for its antibacterial and antifungal activities and more for its anticancer properties (Kalalinia and Karimi-Sani (2017). Thus, our study provides new data with regards to its antimicrobial properties and show that solamargine plays a key role in the pharmacological action of *S. mammosum*. Through LC-HRMS and data analyses of compounds from *S. mammosum* extract, seven compounds were annotated, among which four metabolites that matched with flavonoid compounds: kaempferol (*m/z* 285.0396), sorbifolin (*m/z* 299.0553), tobaflavone E (*m/z* 347.0976) and rutin (*m/z* 609.1449). Flavonoids are associated with a broad spectrum of health-promoting effects and are indispensable components in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications (Panche et al., 2016). An important effect of flavonoids is the scavenging of oxygen-derived free radicals (Nijveldt et al., 2001). Studies about flavonoids reported that several of these compounds exhibit anti-oxidant, antitumoral, anti-inflammatory and antimicrobial activities including antifungal, antiviral, and antibacterial effects (Middleton, 1998; Nijveldt et al., 2001). Kaempferol, which is one of most representative natural flavonol, was reported to have moderate activity on *C. albicans* strains with values of MIC between 128 and 441 μg mL^{-1} (449–1547 μM) (Seleem et al., 2017; Shao et al., 2016), but to be inactive against *P. aeruginosa* (MIC > 1000 μg mL^{-1}) (Adamczak et al., 2019a, b). Sorbifolin, a flavone previously isolated from *Astragalus trimestris* L. (Fabaceae) displayed mild antibacterial activity on *Escherichia coli* and *C. albicans* with MIC value of 125 μg mL^{-1} (417 μM) (El-Hawiet et al., 2010). Plants containing rutin (quercetin-3-O-rutinoside), a flavonol glycoside, are traditionally used as antimicrobial, antiarthritic or antiallergic (Sharma et al., 2016). Nonetheless, these compounds displayed lack of activities on *C. albicans* (Han, 2009; Tempesti et al., 2011) and *P. aeruginosa* (Lou et al., 2015) with MIC > 1000 μg mL^{-1}. Another flavonoid annotated was tobaflavone E, that expressed activity on virus such as TMV (Tobacco Mosaic Virus) with 35.3 ± 3.2% of inhibition (Miao et al., 2015). TMV is one of the most damaging plant virus, causing significant yield losses in crop

production worldwide (Rybicki, 2015). Moreover, three additional compounds were annotated and placed in the network: ethyl caffeate (*m/z* 207.0658), viarumacid A (*m/z* 851.2234) and solamargine (*m/z* 867.3099). First, ethyl caffeate is a compound previously isolated from *Cnestis palala* (Lour.) Merr. (Connaraceae), which has been tested on *Staphylococcus aureus* and *S. epidermidis* with MIC value at 500 μg mL^{-1} (2.41 mM) against both microorganisms (Dej-adisai et al., 2015). Secondly, viarumacid A, a glucosylated caffeoylquinic acid derivative isolated from *S. viarum* has been showed to display antioxidant activity (Wu et al., 2012). The diversity of class of compounds visualized in the polar extract from *S. mammosum* leaves a door open for further exploration in the laboratory.

5Conclusion

The findings of the present investigation conclude that solamargine is the main active compound against *T. mentagrophytes*, *C. albicans in vitro*. In addition, our data indicate that this compound has a significant effect on *P. aeruginosa* biofilm formation and pyocyanin production under our conditions. Taken together, these findings may help to explain why *S. mammosum* fruits have been traditionally used to treat a variety of fungal infections and respiratory disorders.

Declaration of competing interests

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

CRediT authorship contribution statement

Billy Cabanillas: Writing – original draft, Formal analysis, collected the plant, performed the extraction of S. mammosum fruits and analyzed the data. **François Chassagne:** Writing – original draft, performed the microdilution assay against C. albicans. **Pedro Vásquez-Ocmín:** Writing – original draft, Formal analysis, performed the LC-HRMS and molecular network experiments. **Ali Tahrioui:** Formal analysis, performed the P. aeruginosa pyocyanin production and biofilm formation experiments. **Sylvie Chevalier:** Formal analysis, performed the P. aeruginosa pyocyanin production and biofilm formation experiments. **Marieke Vansteelandt:** Formal analysis, performed the LC-HRMS experiments, All authors read, discussed and agreed on the final manuscript. **Asih Triastuti:** Formal analysis, performed the microdilution assay against C. albicans. **Carlos A. Amasifuen Guerra:** identified the plant. **Nicolas Fabre:** Formal analysis, performed the LC-HRMS experiments, All authors read, discussed and agreed on the final manuscript. **Mohamed Haddad:** Writing – original draft, Formal analysis, conceived, designed, performed the experiments (plant collection, bioautography-TLC-HRMS, microdilution assay against T. mentagrophytes, isolation and structure elucidation of Solamargine, LC-HRMS).

Acknowledgments

The authors acknowledge the French National Research Institute for Sustainable Development and the Federal University of Toulouse for research support, the infrastructure, incentive, and collaboration. Besides, the authors acknowledge the Instituto de Investigaciones de la Amazonia Peruana (IIAP) and especially Ms Elsa Rengifo and Dr Kember Mejia for the access to the botanical garden in Allpahuayo Mishana.

References

Acevedo-Rodríguez, P., Strong, M.T., 2008. Floristic richness and affinities in the west indies. Bot. Rev. 74, 5–36. https://doi.org/10.1007/s12229-008-9000-1.

Adamczak, A., Ożarowski, M., Karpiński, T.M., 2019a. Antibacterial activity of some flavonoids and organic acids widely distributed in plants. J. Clin. Med. 9 (1), 109. https://doi.org/10.3390/jcm9010109.

Adamczak, A., Ożarowski, M., Karpiński, T.M., 2019b. Antibacterial activity of some flavonoids and organic acids widely distributed in plants. J. Clin. Med. 9, 109. https://doi.org/10.3390/jcm9010109.

Arnason, T., Uck, F., Lambert, J., Hebda, R., 1980. Maya medicinal plants of san Jose Succotz, Belize. J. Ethnopharmacol. 2, 345–364. https://doi.org/10.1016/s0378-8741(80)81016-6.

Ayala Flores, F., 1984. Ethnobotany in the neotropics. In: Advances in economic botany, 1, pp. 1–8.

Burger, T., Mokoka, T., Fouché, G., Steenkamp, P., Steenkamp, V., Cordier, W., 2018. Solamargine, a bioactive steroidal alkaloid isolated from Solanum aculeastrum induces non-selective cytotoxicity and P-glycoprotein inhibition. BMC Compl. Altern. Med. 18 (1), 137. https://doi.org/10.1186/s12906-018-2208-7.

Burkill, I.H., 1966. A dictionary of the economic products of the Malay Peninsula. Revised reprint. 2 vols. Ministry of Agriculture and Co-operatives, Kuala Lumpur. vol. 1 (A–H), pp. 1–1240, vol 2 (I–Z), pp 1241–2444.

Caceres, A., Alvarez, A.V., Ovando, A.E., Samayoa, B.E., 1991. Plants used in Guatemala for the treatment of respiratory diseases. 1. Screening of 68 plants against gram-positive bacteria. J. Ethnopharmacol. 31 (2), 193–208. https://doi.org/10.1016/0378-8741(91)90005-x.

Caldwell, C.C., Chen, Y., Goetzmann, H.S., Hao, Y., Borchers, M.T., Hassett, D.J., Young, L.R., Mavrodi, D., Thomashow, L., Lau, G.W., 2009. Pseudomonas aeruginosa exotoxin pyocyanin causes cystic fibrosis airway pathogenesis. Am. J. Pathol. 175 (6), 2473–2488. https://doi.org/10.2353/ajpath.2009.090166.

Chaiyasit, D., Choochote, W., Rattanachanpichai, E., Chaithong, U., Chaiwong, P., Jitpakdi, A., Tippawangkosol, P., Riyong, D., Pitasawat, B., 2006. Essential oils as potential adulti- cides against two populations of Aedes aegypti, the laboratory and natural field strains, in Chiang Mai province, northern Thailand. Parasitol. Res. 99 (6), 715–721. https://doi.org/10.1007/s00436-006-0232-x.

CLSI, 2008. In: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi ; Approved Standard-2nd Edition (MA38–A2). Clinical and Laboratory Standards Institute, Wayne, PA.

Crommett, M.K., 2011. Dominican medicinal plant inventory. http://www.prhdr.org/docs/Dominican%20Medicinal%20Plants.pdf.

DeFilipps, R.A., Maina, S.L., Crepin, J., 2004. Medicinal Plants of the Guianas (Guyana, Surinam, French Guiana). Department of Botany, National Museum of Natural History. Smithsonian Institution, Washington, DC.

Dej-adisai, S., Tinpun, K., Wattanapiromsakul, C., Keawpradub, N., 2015. Bio-activities and phytochemical investigation of *cnestis palala* (lour.) Merr. African Journal of Traditional. Complement. Alternative Med. 12, 27–37. https://doi.org/10.4314/ajtcam.v12i3.3.

El-Hawiet, A., Toaima, S.M., Asaad, A., Radwan, M., El-Sebakhy, N., 2010. Chemical constituents from Astragalus annularis Forssk. and A. trimestris L., Fabaceae. Revista Brasileira De Farmacognosia 20, 860–865. https://doi.org/10.1590/S0102-695X2010005000047.

Feunang, Y.D., Eisner, R., Knox, C., Chepelev, L., Hastings, J., Owen, G., Fahy, E., Steinbeck, C., Subramanian, S., Bolton, E., Greiner, R., Wishart, D.S., 2016. ClassyFire: automated chemical classification with a comprehensive computable taxonomy. J. Cheminf. 8 https://doi.org/10.1186/s13321-016-0174-y.

Flores, F.A., 1984. Notes on some medicinal and poisonous plants of Amazonian Peru. Adv. Econ. Bot. 1, 1–8. Ethnobotany in the Neotropics (18 September 1984).

Grieve, M., 1971. A Modern Herbal, 2. Penguin, p. 919. Dover publications, Dover/New York.

Hajdu, Z., Hohmann, J., 2012. An ethnopharmacological survey of the traditional medicine utilized in the community of Porvenir, Bajo Paraguá Indian Reservation, Bolivia. J. Ethnopharmacol. 139, 838–857. https://doi.org/10.1016/j.jep.2011.12.029.

Han, Y., 2009. Rutin has therapeutic effect on septic arthritis caused by Candida albicans. Int. Immunopharm. 9, 207–211. https://doi.org/10.1016/j.intimp.2008.11.002.

Hancock, R., Carey, A.M., 1979. Outer membrane of Pseudomonas aeruginosa: heat- and 2-mercaptoethanol-modifiable proteins. J. Bacteriol. 140, 902–910. https://doi.org/10.1128/JB.140.3.902-910.1979.

Hanelt, P., Buttner, R., Mansfeld, R., 2001. Mansfeld's Encyclopedia of Agricultural and Horticultural Crops (Except Ornamentals). Springer, Berlin, Germany.

Hsu, A.R., Hsu, J.W., 2012. Topical review: skin infections in the foot and ankle patient. Foot Ankle Int. 33 (7), 612–619. https://doi.org/10.3113/FAI.2012.0612.

Jovel, E.M., Cabanillas, J., Towers, G.H.N., 1996. An ethnobotanical study of the traditional medicine of the Mestizo people of Suni Miraño, Loreto, Peru. J. Ethnopharmacol. 53 (3), 149–156. https://doi.org/10.1016/0378-8741(96)01437-7.

Kalalinia, F., Karimi-Sani, I., 2017. Anticancer properties of solamargine: a systematic review. Phytother Res. 31, 858–870. https://doi.org/10.1002/ptr.5809.

Lans, C., Harper, T., Georges, K., Bridgewater, E., 2001. Medicinal and ethnoveterinary remedies of hunters in Trinidad. BMC Complement. Altern. Med. 1 (10) https://doi.org/10.1186/1472-6882-1-10.

Lau, G.W., Ran, H., Kong, F., Hassett, D.J., Mavrodi, D., 2004. Pseudomonas aeruginosa pyocyanin is critical for lung infection in mice. Infect. Immun. 72 (7), 4275–4278. https://doi.org/10.1128/IAI.72.7.4275-4278.2004.

Levin, R.A., Watson, K., Bohs, L., 2005. A four-gene study of evolutionary relationships in Solanum section Acanthophora. Am. J. Bot. 92, 603–612. https://doi.org/10.3732/ajb.92.4.603.

Lim, T.K., 2013. Solanum linearifolium. In: Edible Medicinal and Non-medicinal Plants. Springer, Dordrecht.

Lou, Z., Tang, Y., Song, X., Wang, H., 2015. Metabolomics-based screening of biofilm-inhibitory compounds against Pseudomonas aeruginosa from burdock leaf. Molecules 20, 16266–16277. https://doi.org/10.3390/molecules200916266.

Luziatelli, G., Sørensen, M., Theilade, I., Mølgaard, P., 2010. Asháninka medicinal plants: a case study from the native community of Bajo Quimiriki, Junín, Peru. J. Ethnobiol. Ethnomed. 6, 21. https://doi.org/10.1186/1746-4269-6-21. https://doi.org/10.1186/1746-4269-6-21.

Miao, M.M., Li, L., Shen, Q.P., Liu, C.B., Li, Y.K., Zhang, T., Zhang, F.M., He, P., Wang, K. M., Zhu, R.Z., Chen, Y.K., Yang, G.Y., 2015. Anti-TMV activity flavones from the leaves of Yunnan local air cured tobacco. Fitoterapia 103, 260–264. https://doi.org/10.1016/j.fitote.2015.04.014.

Middleton Jr., E., 1998. Effect of plant flavonoids on immune and inflammatory cell function. Adv. Exp. Med. Biol. 439, 175–182. https://doi.org/10.1007/978-1-4615-5335-9_13.

Morin, C.D., Déziel, E., Gauthier, J., Levesque, R.C., Lau, G.W., 2021. An organ system-based synopsis of Pseudomonas aeruginosa virulence. Virulence 12 (1), 1469–1507. https://doi.org/10.1080/21505594.2021.1926408.

Muñoz, V., Sauvain, M., Bourdy, G., Callapa, J., Rojas, I., Vargas, L., Tae, A., Deharo, E., 2000. The search for natural bioactive compounds through a multidisciplinary approach in Bolivia. Part II. Antimalarial activity of some plants used by Mosetene Indians. J. Ethnopharmacol. 69, 139–155. https://doi.org/10.1016/s0378-8741(99)00096-3.

Nijveldt, R.J., van Nood, E., van Hoorn, D.E., Boelens, P.G., van Norren, K., van Leeuwen, P.A., 2001. Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr. (74), 418–425. https://doi.org/10.1093/ajcn/74.4.418, 2001.

O'Toole, G.A., 2011. Microtiter dish biofilm formation assay. JoVE 47, 2437. https://doi.org/10.3791/2437.

Olivon, F., Elie, N., Grelier, G., Roussi, F., Litaudon, M., Touboul, D., 2018. MetGem software for the generation of molecular networks based on the t-SNE algorithm. Anal. Chem. 90, 13900–13908. https://doi.org/10.1021/acs.analchem.8b03099.

Oviedo, R., Herrera, P., Caluff, M.G., Regalado, L., Ventosa, I., 2012. Lista nacional de especies de plantas invasoras y potencialmente invasoras en la República de Cuba -2011. Plantas invasoras en Cuba, Bissea.

Panche, A.N., Diwan, A.D., Chandra, S.R., 2016. Flavonoids: an overview. J. Nutr. Sci. 5, e47. https://doi.org/10.1017/jns.2016.41.

Patel, K., Singh, R., Patel, D., 2013. Medicinal significance, pharmacological activities, and analytical aspects of solasodine: a concise report of current scientific literature. J. Acute Dis. 2, 92–98. https://doi.org/10.1016/S2221-6189(13)60106-7.

Pier, 2014. Pacific Islands Ecosystems at Risk. HEAR, University of Hawaii, Honolulu, USA. http://www.hear.org/pier/index.html.

Pinedo, M., Rengifo, E., Cerruti, T., 1997. Plantas medicinales de uso popular en la Amazonía Peruana, studio de su uso y cultivo. Instituto de Investigaciones de la Amazonía Peruana, p. 315. Iquitos, Peru).

Pluskal, T., Castillo, S., Villar-Briones, A., Oresic, M., 2010. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinf. 11, 395. https://doi.org/10.1186/1471-2105-11-395.

Polesna, L., Polesny, Z., Clavo, M.Z., Hansson, A., Kokoska, L., 2011. Ethnopharmacological inventory of plants used in coronel Portillo province of Ucayali department, Peru. Pharm. Biol. 49, 125–136. https://doi.org/10.3109/13880209.2010.504927.

Rahalison, L., Hamburger, M., Hostettmann, K., Monod, M., Frenk, E., 1991. A bioautographic agar overlay method for the detection of antifungal compounds from higher plants. Phytochem. Anal. 2, 199–203. https://doi.org/10.1002/pca.2800020503.

Rinaldi, M.G., 2000. Dermatophytosis: epidemiological and microbial update. J. Am. Acad. Dermatol. 43 (5 Suppl. l), s120–s124. https://doi.org/10.1067/mjd.2000.110378.

Roddick, J.G., Rijnenberg, A.L., 1986. Effect of steroidal glycoalkaloids of the potato on the permeability of liposome membranes. Physiol. Plantarum 68, 436–440. https://doi.org/10.1111/j.1399-3054.1986.tb03378.x.

Roumy, V., Garcia-Pizango, G., Gutierrez-Choquevilca, A.L., Ruiz, L., Jullian, V., Winterton, P., Fabre, N., Moulis, C., Valentin, A., 2007. Amazonian plants from Peru used by Quechua and Mestizo to treat malaria with evaluation of their activity. J. Ethnopharmacol. 112, 482–489. https://doi.org/10.1016/j.jep.2007.04.009.

Rybicki, E.P., 2015. A Top Ten list for economically important plant viruses. Arch. Virol. 160, 17–20. https://doi.org/10.1007/s00705-014-2295-9.

Sawariam, I., 1986. Studi Kandungan Buah Solanum Mammosum L, Skripsi (BSc Thesis). Fakultas Farmasi Universitas Airlangga, Surabaya.

Seleem, D., Pardi, V., Murata, R.M., 2017. Review of flavonoids: a diverse group of natural compounds with anti-Candida albicans activity in vitro. Arch. Oral Biol. 76, 76–83. https://doi.org/10.1016/j.archoralbio.2016.08.030.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504. https://doi.org/10.1101/gr.1239303.

Shao, W., Liu, H., Wang, S., Wu, J., Huang, M., Min, H., Liu, X., 2016. Controlled release and antibacterial activity of tetracycline hydrochloride-loaded bacterial cellulose composite membranes. Carbohydr. Polym. 145, 114–120. https://doi.org/10.1016/j.carbpol.2016.02.065.

Sharma, P., Kalita, M.C., Thakur, D., 2016. Broad spectrum antimicrobial activity of forest-derived soil actinomycete, Nocardia sp. PB-52. Front. Microbiol. 7 (347) https://doi.org/10.3389/fmicb.2016.00347.

Space, J., Flynn, T., 2002. Report to the Government of the Cook Islands on Invasive Plant Species of Environmental Concern. USDA Forest Service, Honolulu, USA. Pacific southwest research station, Institute of Pacific Islands forestry, 146.

Stuart, G.U., 2010. Philippine alternative medicine. In: Manual of Some Philippine Medicinal Plants. http://www.stu-artxchange.org/OtherHerbals.html.

Tahrioui, A., Ortiz, S., Azuama, O.C., Bouffartigues, E., Benalia, N., Tortuel, D., Maillot, O., Chemat, S., Kritsanida, M., Feuilloley, M., Orange, N., Michel, S., Lesouhaitier, O., Cornelis, P., Grougnet, R., Boutefnouchet, S., Chevalier, S., 2020. Membrane-interactive compounds from Pistacia lentiscus L. Thwart Pseudomonas aeruginosa virulence. Front. Microbiol. 11 (1068) https://doi.org/10.3389/fmicb.2020.01068.

Tarigan, P., 1980. Sapogenin Steroid. Perbit Alumni, Bandung, pp. 96–103.

Telek, L., 1977. Determination of solasodine in fruits of solarium species. J. Pharmaceut. Sci. 66, 699–702. https://doi.org/10.1002/jps.2600660523.

Tempesti, T.C., Álvarez, M.G., Araújo, M.F., Júnior, F.E., Carvalho, M.G., Durantini, E., 2011. Antifungal activity of a novel quercetin derivative bearing a trifluoromethyl group on Candida albicans. Med. Chem. Res. 21, 2217–2222. https://doi.org/10.1007/s00044-011-9750-x.

Vásquez, R., 1997. Flórula de las reservas biológicas de Iquitos. Missouri Botanical Garden, Perú, p. 1046p. Iquitos, Peru.

Vega, M., 2001. Etnobotánica de la amazonía peruana, 1ra. Edicion. Ediciones, Abya-Yala, p. 166. Quito, Ecuador.

Wiart, C., Mogana, S., Khalifah, S., Mahan, M., Ismail, S., Buckle, M., Narayana, A.K., Sulaiman, M., 2004. Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. Fitoterapia 75, 68–73. https://doi.org/10.1016/j.fitote.2003.07.013.

Wu, S.B., Meyer, R.S., Whitaker, B.D., Litt, A., Kennelly, E.J., 2012. Antioxidant glucosylated caffeoylquinic acid derivatives in the invasive tropical soda apple, Solanum viarum. J. Nat. Prod. 75, 2246–2250. https://doi.org/10.1021/np300553t.