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

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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 $\mu\text{g}\cdot\text{mL}^{-1}$) and anti-*Candida albicans* (MIC = 64 $\mu\text{g}\cdot\text{mL}^{-1}$) 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 $\mu\text{g}\cdot\text{mL}^{-1}$) and remained fairly consistent regardless of the concentration. In addition, solamargine reduced pyocyanin production by about 20% at concentrations of 12.5 and 50 $\mu\text{g}\cdot\text{mL}^{-1}$. 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.

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

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-Rodriguez and Strong, 2012), 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 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 2000; DeFilipps et al. 2004; Chaiyasit 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 hours 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 μ L of the amphotericin B stock solution and 5 μ L of *S. mammosum* extract at the concentrations of 10, 20 and 30 mg.mL⁻¹ were spotted on TLC plates. Standard mobile phases CHCl₃/MeOH/H₂O (65:40:10, v/v/v) and CHCl₃/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 μ L.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 CHCl₃/MeOH/H₂O (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 McFarland 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 ($10 \text{ mg}\cdot\text{mL}^{-1}$ in DMSO) were serially diluted in RPMI 1640 medium so that 8 concentrations in the range of $4\text{-}512 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$ 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 μ L 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 deconvoluted 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 Figure 1. Solamargine (**1**, Figure 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 $\mu\text{g.mL}^{-1}$) when compared to Amphotericin B (100% inhibition at 8 $\mu\text{g.mL}^{-1}$) whereas solamargine exhibited a moderate activity against the three strains (MIC = 64 $\mu\text{g.mL}^{-1}$).

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 $\mu\text{g.mL}^{-1}$, 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 $\mu\text{g.mL}^{-1}$ (Figure 3a). However, no impact was observed at low concentrations. Solamargine also led to a significant reduction of about 20% of the biofilm formation (Figure 3b). This effect was observed for a very low concentration (1.6 $\mu\text{g.mL}^{-1}$) 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 $\mu\text{g.mL}^{-1}$.

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 (Figure 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 *chufcha* (Pinedo et al., 1997; Vasquez, 1997; Vega, 2001) is commonly used in Peru to treat mycosis and scabies (Roumy et al., 2020), 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., 2020 ; 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 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 $\mu\text{g.mL}^{-1}$ 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 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 pneumoniae*). *P. aeruginosa* is a pathogenic gram-positive bacteria responsible of respiratory 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 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 metabolites which are both highly toxic to humans but medicinally useful. It is a source of solasodine (Telek et al., 1977; Roddick, 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, cardiogenic, antispermatogenic, 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 – 441 $\mu\text{g}\cdot\text{mL}^{-1}$ (449-1547 μM) (Seleem et al., 2017; Shao et al., 2016), but to be inactive against *P. aeruginosa* (MIC > 1000 $\mu\text{g}\cdot\text{mL}^{-1}$) (Adamczak et al., 2020). 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 $\mu\text{g}\cdot\text{mL}^{-1}$ (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., 2013). Nonetheless, these compounds displayed lack of activities on *C. albicans* (Han, 2009; Tempesti et al., 2012) and *P. aeruginosa* (Lou et al., 2015) with MIC > 1000 $\mu\text{g}\cdot\text{mL}^{-1}$. 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 $\mu\text{g}\cdot\text{mL}^{-1}$ (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.

Conclusion

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 interest

The authors declare that there are no conflicts of interest.

Author contributions

Mohamed Haddad: conceived, designed, performed the experiments (plant collection, bioautography-TLC-HRMS, microdilution assay against *T. mentagrophytes*, isolation and structure elucidation of Solamargine, LC-HRMS), analyzed the data and wrote the original manuscript; **Billy Cabanillas** collected the plant, performed the extraction of *S. mammosum* fruits and analyzed the data; **François Chassagne** performed the microdilution assay against *C. albicans*, analyzed the data and wrote the manuscript; **Pedro Vásquez-Ocmín** performed the LC-HRMS and molecular network experiments, analyzed the data and wrote the

manuscript; **Ali Tahrioui** and **Sylvie Chevalier** performed the *P. aeruginosa* pyocyanin production and biofilm formation experiments and analyzed the data ; **Asih Triastuti** performed the microdilution assay against *C. albicans* and analyzed the data; **Carlos A. Amasifuen Guerra** identified the plant; **Marieke Vansteelandt** and **Nicolas Fabre** performed the LC-HRMS experiments and analyzed the data. All authors read, discussed and agreed on the final manuscript.

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Legends

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

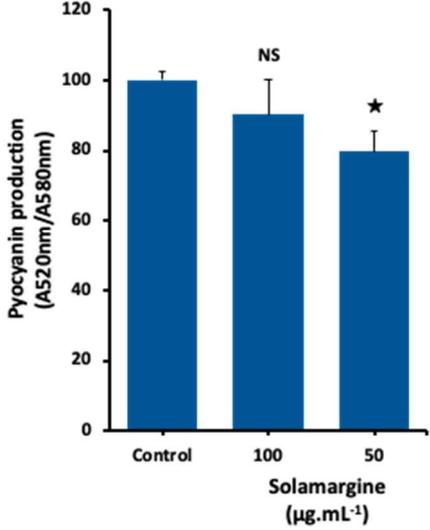
Figure 2. Structure of solamargine 1

Figure 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), $p\geq 0.05$.

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

Figure 3.

(a)



(b)

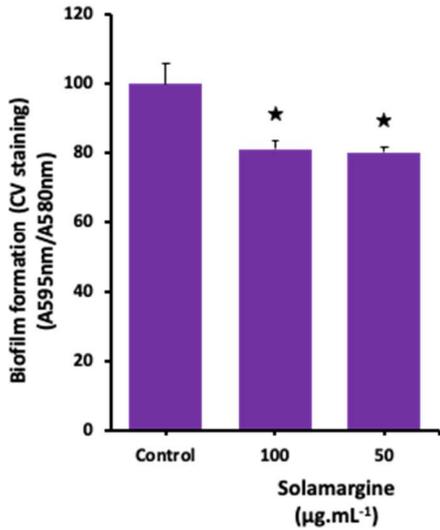
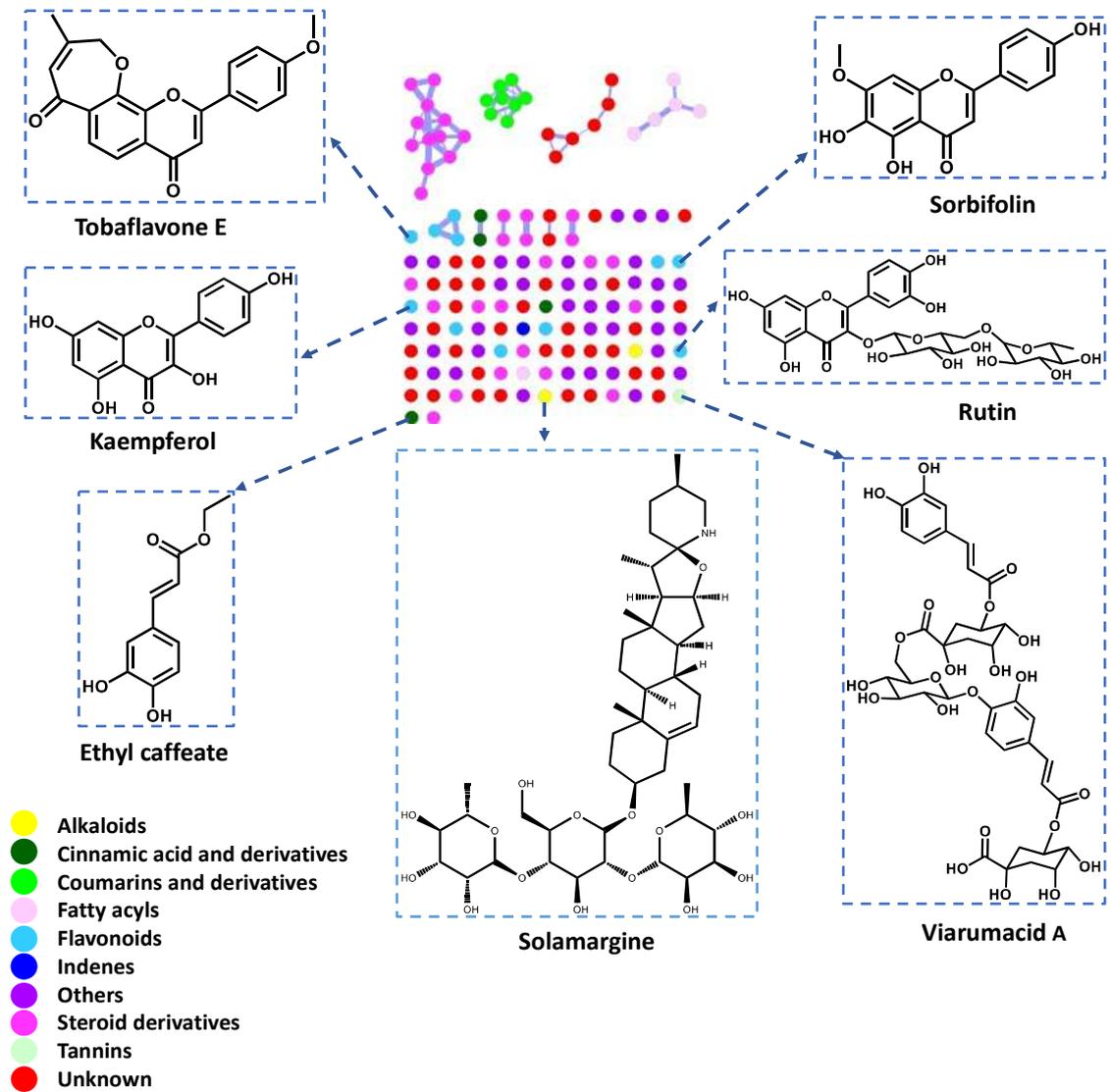


Figure 4.



Legends

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

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

Table 1.

	MIC ($\mu\text{g.mL}^{-1}$)		
	<i>T. mentagrophytes</i>	<i>C. albicans</i> 90028	<i>C. albicans</i> 10231
TDV crude fruit extract	256	256	256
Solamargine	64	64	64

Table 2.

<i>m/z</i> [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

Graphical abstract

