Eur Food Res Technol (2017) 243:2155–2161
DOI 10.1007/s00217-017-2918-y

ORIGINAL PAPER

Identification and antimicrobial activity of the sesquiterpene lactone mixture extracted from *Smallanthus sonchifolius* dried leaves

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Received: 31 January 2017 / Revised: 12 May 2017 / Accepted: 19 May 2017 / Published online: 30 May 2017

Abstract The present study aimed to identify and screen the antibacterial activity of a mixture of sesquiterpene lactones (SLs) obtained from the dichloromethane extract of *Smallanthus sonchifolius* leaves. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella enterica* strains were used as bacterial tests. The identification and quantification of compounds were established by GC/MS and ^{1}H, ^{13}C 2D NMR spectroscopic analysis. The antimicrobial activity was measured by the disk diffusion method and minimal inhibitory concentration assay (MIC). In vitro antimicrobial assays showed that the mixture of SLs found (uvedalin and enhydrin) displays poor antibacterial activity against the Gram-negative bacteria and appreciable antibacterial properties against the Gram-positive bacterial strain tested when 90 µg of the SLs mixture per disk was used. The MIC determination against *S. aureus* revealed that a concentration of 750 µg of SLs mixture mL^{-1} should be necessary to inhibit the strain. These results indicate that the SLs mixture of enhydrin and uvedalin from yacon leaves presents promising antibacterial properties against *S. aureus* and apparent lack of activity against the Gram-negative bacterial strains tested at the concentrations applied.

Keywords Yacon · Gram-positive bacteria · *Staphylococcus aureus* · GC–MS · NMR

Introduction

Yacon (*Smallanthus sonchifolius*) (Poepp.) H. Rob is a perennial herb from the *Asteraceae* family, originated from the Andes and being grown in several regions of the world [1]. The plant root is usually consumed raw or in the form of syrups, juices, and marmalades, while the leaves are used in infusions to make teas [2]. The extract of yacon leaves has a variety of phytochemicals that present important biological activities, including the antioxidant [3, 4], anti-inflammatory [5], and antimicrobial ones [6]. Recent studies demonstrated that the oral intake of the yacon hydroethanolic extract was beneficial to rats in the sense of reducing blood glucose levels without presenting liver damages [7].

Smallanthus sonchifolius leaves are rich in sesquiterpene lactones (SLs), stored inside glandular trichomes on the foliar surface [5, 8]. Sesquiterpene lactones consist of natural terpenoid compounds, secondary metabolites present in some *Asteraceae* (among others) family plants. These metabolites are specially known for their antitumoral and anti-inflammatory activities [9–11]. Topical antiedematous in vivo activity was observed in extracts from yacon leaves, and this activity was attributed to the anti-inflammatory properties of the SLs [5, 6]. De Ford et al. [10] verified that SLs exert poor cytotoxic effects on the blood mononuclear cells of healthy human subjects, but a strong cytotoxicity in leukemia and pancreas cancer cells. Other studies have also demonstrated that yacon displays antibacterial

Electronic supplementary material The online version of this article (doi:10.1007/s00217-017-2918-y) contains supplementary material, which is available to authorized users.

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and antifungal effects [12, 13]. The antimicrobial activity of yacon organic extracts against methicillin-resistant *Staphylococcus aureus* (MRSA) was proved to be effective when mixed with the ampicillin or oxacillin antibiotics, showing a synergistic effect [12]. These activities have been attributed to the SLs, such as enhydrin, uvedalin, sonchifolin, and polymatin B, which are naturally present in the leaves [13, 14]. In a contribution to this filed, Lin et al. [6] also reported the antimicrobial potential of the SLs 8β-methacryloyloxymelampolid-14-oic acid methyl ester and 8β-tigloyloxymelampolid-14-oic acid methyl ester from yacon leaves against *Bacillus subtilis* and *Pericularya oryzae*. However, as most researches aim at verifying the potential of the single effect of SLs or their combination with (semi)synthetic antibiotics against microbial strains, further studies are still needed toward the assessment of the potential of real SL combinations found in different crops. Thus, to better understand the potential of the sesquiterpene lactones found in yacon leaves as antimicrobial agents, this work was aimed to evaluate the antimicrobial activity of the SLs mixture (enhydrin/uvedalin) extracted from *S. sonchifolius* leaves against strains of *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 15442), *S. aureus* (ATCC 6538), and *Salmonella enterica* (ATCC 14028).

Materials and methods

Plant material preparation

Leaves of *S. sonchifolius* were harvested during autumn, in São José dos Pinhais, a city near Curitiba, Paraná state, Brazil (coordinates: 25°37′14″ latitude, 49°07′40″W, altitude of 906 m). A voucher specimen was deposited in the herbarium of the Federal University of Paraná.

Extraction and isolation

For the isolation and characterization of the sesquiterpene lactones present in *S. sonchifolius* leaves, the procedures described by Schorr and da Costa [15] and Genta et al. [16] were followed applying some modifications. Yacon leaves (150 g) were completely air-dried (until constant weight) in a convection oven at 40 °C, followed by a dichloromethane extraction (J.T. Backer®, USA) (1 L) in a glass beaker (2 L) for 15 min. The extract obtained was filtered through filter paper, and the solvent evaporated under reduced pressure at 40 °C, resulting in 4.49 g of crude residue, which was dissolved in methanol at 40–45 °C (35 mL). After the residue solubilization, water was added dropwise (15 mL) to precipitate waxes. The waxes were removed by filtration through filter paper, and the filtrate was evaporated under reduced pressure. The dewaxed residue was re-dissolved in methanol (9 mL) and stored in freezer at −20 °C for 4 days. A crystalline precipitate was obtained (20 mg of SLs), which was separated by decantation and washed with 3 × 1 mL aliquots of cold diethyl ether (Merck®, Germany). The precipitate was then prepared for identification and quantification by GC/MS and nuclear magnetic resonance assays.

Nuclear magnetic resonance analysis (NMR)

The ^{1}H NMR and 2D NMR spectra were recorded on a Bruker AVANCE III 600 spectrometer equipped with a 5-mm quadrinuclear inverse probe (^{1}H, ^{13}C, ^{15}N e ^{31}P), operating at 600.13 MHz on ^{1}H and 150 MHz on ^{13}C. The ^{13}C and DEPT NMR spectra were recorded on a Bruker AVANCE III 400 spectrometer equipped with a 5-mm multinuclear BBO probe. Standard pulse sequences were used for homo- and heteronuclear correlation experiments. The spectra were registered at room temperature (295 K). Data were processed with NMR Processor (v.12.01.33929) software from ACD/Labs. The samples were dissolved in deuterated chloroform ($CDCl_3$), with tetramethylsilane (TMS) as internal standard (CIL, USA).

Gas chromatography and mass spectrometry (GC/MS)

The GC/MS analyses were performed using a Varian® 320-MS selective quadrupole mass detector coupled to a Varian® GC-450 Gas Chromatograph fitted with a CP Sil 8CB column (30 m × 0.25 mm i.d. × e 0.25 µm film thickness). The following conditions were employed to analyze the sesquiterpene lactones: ionization energy of 70 eV; selective mass detector temperature, GC/MS interphase, ion source and injector temperature were maintained at 280, 250, and 280 °C, respectively; injection volume of 1 µL (split ratio 1:20); and helium as carrier gas at 1.2 mL min^{-1}. The oven temperature was programmed as follows: from 60 °C to 250 °C at 10 °C min^{-1} and from 250 to 300 °C at 5 °C min^{-1}, holding at 300 °C for 5 min. The samples were injected in methylene chloride. Sesquiterpene lactones identification was made by comparing the experimental mass spectra with a commercial gas chromatography–mass spectra library (National Institute of Standards and Technology—NIST) and with mass spectra reported in the literature [17].

Antimicrobial activity

In order to determine the antimicrobial activity of the sesquiterpene lactones, the Gram-positive and Gram-negative bacteria used as indicators were: *E. coli* (ATCC 11229), *P.*

aeruginosa (ATCC 15442), *S. aureus* (ATCC 6538), and *S. enterica* (ATCC 14028).

Agar diffusion

A paper disk method was used for the determination of antimicrobial activities of the sesquiterpene lactones of *S. sonchifolius* leaves according to the method described by Bauer et al. [18]. The sterile antibiotic disk assays (Laborclin®, Brazil) were loaded with a known sesquiterpene lactone amount dissolved in 100% dimethyl sulfoxide (DMSO) (Merck®, Germany) and then dried for 2 h under vacuum to remove the solvent. A bacterial culture (Mcfarland standard No. 0.5) was used to lawn Mueller–Hinton (Difco®) agar plates using a sterile swab. The disks impregnated with different amounts of sesquiterpene lactones (10–90 µg) were placed on a Mueller–Hinton agar surface. The test plates comprised of two positive control disks (ampicillin 10 µg and ceftazidime 30 µg), a negative control disk (DMSO 100%), and three treated disks. The plates were incubated at 37 °C for 18–24 h. The experiments were repeated in the absence and in the presence of light (4000 lx). After incubation, the diameter of the inhibition zones was measured using a caliper and recorded. The average of three measurements was adopted to ensure the measurements reliability.

Minimum inhibitory concentration (MIC)

The MIC of the sesquiterpene lactones was determined by the broth microdilution method according to the National Committee for Clinical Laboratory Standards M7-A7 guidelines through the Clinical and Laboratory Standards Institute [19]. Serial dilutions of the sesquiterpene lactones were prepared in 96-well plates with concentrations between 1/2 and 1/250. Fresh bacterial strain cultures were inoculated into each well in order to achieve a final inoculum of 5×10^5 colony-forming units (CFU mL^{-1}) or 5×10^4 CFU per well. After 24 h of incubation at 37 °C, each well was examined for cellular growth and compared to the control. The positive control consisted of a bacterial suspension added to a well containing Mueller–Hinton broth with no sesquiterpene lactones. The MIC was defined as the lowest concentration of an antimicrobial agent that completely inhibits the growth of organisms in the well, detected by naked eye.

Results and discussion

Identification of sesquiterpene lactones in *Smallanthus sonchifolius* leaves

The mixture of sesquiterpene lactones was obtained as a crystalline precipitated from the extract of *S. sonchifolius* leaves. The GC/MS analysis confirmed the presence of two compounds (named **1a** and **1b**) with *m/z* 272 [M^+] and *m/z* 256 [M^+], respectively.

The ^{1}H NMR of compound **1a** indicates five methyl groups, at δ_H 1.17 (d, $J = 5.5$, H-4′), 1.71 (s, H-15), 1.45 (s, H-5′), 2.05 (s, H-9′), and 3.83 (s, H-14′), one exocyclic olefinic CH_2 group at δ_H 5.84 (d, $J = 3.2$, H-13_{trans}) and 6.33 (d, $J = 3.6$, H-13_{cis}), and one olefinic CH group at δ_H 7.15 (dd, $J = 10.5$, 7.6, H-1). All hydrogen signals including the δ 2.68 (d, $J = 9.7$ Hz, H-5); 4.28 (dd, $J = 9.7 \times 2$ Hz, H-6); 5.87 (d, $J = 8.6$ Hz, H-9); 6.71 (dd, $J = 8.4$, 1.05 Hz, H-8) are in accordance with [13, 20, 21].

For compound **1b**, the methine hydrogens were observed at δ_H 2.79 (dtd, $J = 9.7$, 3.3×2 Hz, H-7); 4.96 (dd, $J = 10.4$; 0.7, H-5); 5.11 (dd, $J = 10.1 \times 2$ Hz, H-6); 5.41 (d, $J = 8.4$ Hz, H-9); 6.66 (dd, $J = 8.3$, 1.4 Hz, H-8); and 7.01 (dd, $J = 10.3$, 7.6 Hz, H-1), in accordance with the data obtained by Hong et al. [22] and Ali et al. [23]. Spectroscopic data of the ^{13}C-NMR (DEPT), the ^{1}H, ^{13}C-HSQC assignments, and the (^{1}H-^{13}C)-HMBC correlation for compounds **1a** and **1b** are available as Supplementary Material.

All the evidences support that the mixture of compounds **1a** and **1b** (SLs) present in the extract of *S. sonchifolius* leaves consisted of a mixture of two compounds: enhydrin and uvedalin, respectively (Fig. 1). These compounds were

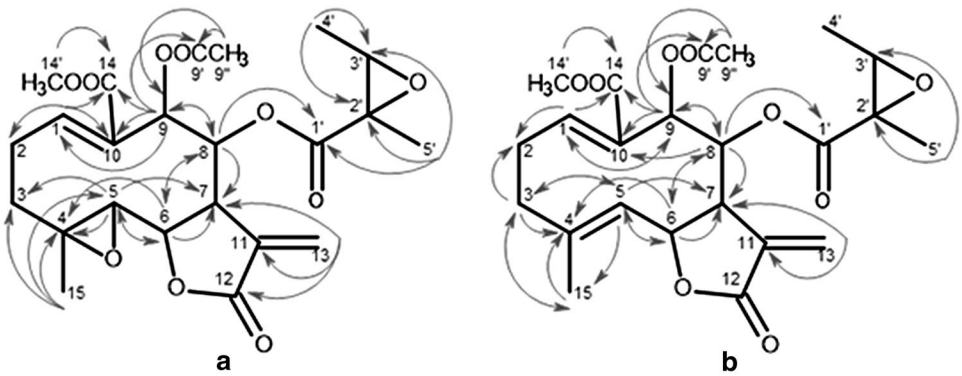

Fig. 1 Chemical structures of **1a** (enhydrin) and **1b** (uvedalin) sesquiterpene lactones

previously identified in extracts of yacon leaves [6, 15–17, 24]. The concentration of each component was determined by the ratio of integrated intensity from double doublet signals at 7.15 ppm (**1a**) and 7.01 ppm (**1b**). For the samples of yacon leaves analyzed, a 3.5:1 (**1a**:**1b**) ratio was found in the extract.

Antimicrobial activity

The evaluation of antimicrobial activity of the SLs found in yacon leaves (enhydrin/uvedalin mixture) showed that *P. aeruginosa* ATCC 15442, *E. coli* ATCC 11220, and *S. enterica* ATCC 14028 strains were not susceptible to concentrations up to 90 µg of the SLs mixture per disk (Table 1). On the other hand, the SLs mixture showed activity against the Gram-positive bacteria *S. aureus* ATCC 6538 at the concentration of 90 µg per disk, presenting a 8.67 ± 0.5 mm zone inhibition (Table 1). Similar results were observed in the work driven by Aliyu et al. [25], where the SLs isolated from *Vernonia brumeoids* showed moderate activity against *S. aureus* ATCC 29213.

The inhibitory effect of SLs is dependent on the molecule type, cell characteristics, and accessibility [26, 27]. In 1991, Gershenzon and Croteau [28] have estimated that about 3500 different sesquiterpene lactones were known until then. As more than twenty years passed by, many other molecules of this type have probably been discovered. The vast amount of SLs and cell types available makes it impossible to determine the exact molecular structures responsible for toxicity and inhibition. Studies in this sense usually evaluate the differences in SLs structures to verify which functional groups may impose higher effects over fixed cell types or strains. Some authors suggest that the antimicrobial activity of SLs depend upon the presence of an α-methylene-γ-lactone group attached to the molecule, although the existence of this structure alone is not the only requisite for the existence of such effect [26, 29, 30]. Other cases involve the analysis of the epoxide group present at the C4/C5 position of the enhydrin molecule (Fig. 1). These studies verified that the epoxides present in the enhydrin molecule enhance the cytotoxicity on cervical cancer cells [31] and CCRF-CEM and MIA-PaCa-2 cells [10]. According to de Ford et al. [10], the double bound between C4 and C5, i.e., the absence of this epoxide group in uvedalin structure, makes its toxicity less pronounced on these kinds of cells. Regarding antifungal activity, Wedge et al. [26] did not find this inhibitory effect attributed to C4/C5 epoxide groups.

Another factor that may impose toxic effects is the angeloyl group presence instead of an epoxide structure in the C8 side chain, which may improve the cytotoxicity effect in cancer cells [10]. However, studying several SL types, Cartagena et al. [30] observed no influence of the nature of C8 or C14 groups on *S. aureus* and other microbial strains. Other authors also stated that low polar SLs presented higher antifungal activity [13, 32].

The relation of polarity with the antimicrobial activity of some sesquiterpene melampolides and sesquiterpene lactones was previously described by Inoue et al. [13] and Barrero et al. [32] who concluded that the least polar compounds showed the greatest activity. Based on the current and previous studies, the lipophilicity of sesquiterpene lactones seems to play a role in their antibacterial activity. Since the chemical composition of the cell walls of the bacteria is highly lipophilic, a moderate to high lipophilicity is essential for good antibacterial activity.

In the case of the uvedalin and enhydrin, their single structural difference remains in the existence of an epoxide group (enhydrin) or an unsaturation (uvedalin) at C4–C5. Lin et al. [6] found that uvedalin presented approximately 39% higher inhibitory activity against *B. subtilis* (Gram-positive) than enhydrin. For this microbial type the absence of the epoxide group favored the inhibitory effect. These authors also attributed an inhibitory effect to the acetoxy group present at the C9 position (Fig. 1) in both molecules.

In the present work, the antimicrobial activity assays showed that the mixture of SLs (enhydrin/uvedalin) extracted from *S. sonchifolius* leaves can affect at least the *S. aureus* ATCC 6538 strain, but may possibly affect other *S. aureus* strains or even inhibit other Gram-positive bacteria. Also, it must be emphasized that although *S. aureus* is a bacterium commonly found in the colonizing microbiota of the skin and mucous membranes of mammals, some strains may behave as opportunistic pathogens, leading to minor

Table 1 Antimicrobial activity (as inhibition zone, mm) of the SLs, and the positive (ampicillin and ceftazidime) and negative controls

Strain	Inhibition zone (mm)				
	DMSO	SLs 10 µg	SLs 90 µg	Ampicillin 10 µg	Ceftazidime 30 µg
Pseudomonas aeruginosa	ND	ND	ND	ND	24.5 ± 3.5
Escherichia coli	ND	ND	ND	23.5 ± 0.7	26.0 ± 1.4
Staphylococcus aureus	ND	ND	8.67 ± 0.57	48.0 ± 1.4	22.5 ± 0.7
Salmonella enterica	ND	ND	ND	26.5 ± 0.7	28.0 ± 1.4

ND antimicrobial activity not detected, *DMSO* negative control, *ampicillin and ceftazidime* positive controls

or severe infection in soft tissues [33]. In this case, the SLs represent promising antibacterial agents. However, due to the great number and variety of existing microorganisms, this field of study is for now little explored and needs further research.

In a similar work, Pak et al. [34] studied the effect of flavonoids, enhydrin, and enhydrin/uvedalin mixture on the aflatoxin B_1 production and the growth of *Aspergillus flavus*. Enhydrin was tested at a maximum of 14 μg mL^{-1} and did not present statistically significant results (~11% reduction), although a concentration-dependent trend was observed in reducing both fungus and aflatoxin content. On the other hand, the enhydrin/uvedalin mixture was capable of decreasing 34% of the *A. flavus* grow and 76% of aflatoxin B_1 production. However, the authors did not specify the concentration of each SL in the mixture. Similarly, Inoue et al. [13] verified the activity of different sesquiterpene lactones against *Pyricularia oryzae* at the concentration of 20–500 ppm. The authors found a highest inhibiting effect with sonchifolin, followed by polymatin B, uvedalin, and finally, enhydrin. At 250 and 50 ppm, uvedalin was able to inhibit 70.2 and 100% of the fungus, while enhydrin was able to reduce only 5.1% for both concentrations. It seems that although enhydrin is present in a larger amount in yacon leaves, its antimicrobial effect may be lower depending on the target microorganism.

Due to the variability, the complete mechanism of action of sesquiterpene lactones on microorganisms is unclear. As sesquiterpene lactones belong to the terpenoids class, we may suggest similar mechanisms of action. Trombetta et al. [35], for example, verified that the menthol, thymol, and linalyl acetate terpenes were able to interfere in the lipid fraction of *S. aureus* ATCC 6538P membrane and disrupt it, causing the leakage of intracellular material. The authors also considered that this effect may be dependent on the terpene characteristics, the membrane lipid composition, and net surface charge. Additionally, Badawy et al. [36] reported that due to the high lipophilicity of bacterial cell walls, moderate to high lipophilic SLs would be essential for improved inhibitory effect.

Another study worth mentioning is that of Joung et al. [12], who evaluated the antimicrobial activity of the methanolic extract of *Smallantus sonchifolius* leaves and its *n*-hexane, ethyl acetate, *n*-butanol and water fractions against methicillin-resistant and methicillin-susceptible *S. aureus* strains. The samples were not dried, and the microbial assessments were performed under the presence (4000 lx) and absence of light. The antimicrobial activity was performed by the disk diffusion method, and no inhibition was found when 50 and 100 μg of extract per disk was applied in the dark. This result may have occurred due to the difference in using an extract (containing several other molecule types, and less SLs) and an enhydrin/uvedalin mixture. The authors have found inhibition zones when light was applied on the plates, and this effect is further discussed in the next section.

The results obtained in this work and gathered from the literature support the existence of an inhibiting effect of the enhydrin/uvedalin mixture on microorganisms. The degree of inhibition should be a function of the organism type and strain, and the type and concentration of each SL.

Minimum inhibitory concentration (MIC)

The antimicrobial activity assays showed that the uvedalin and enhydrin mixture do not inhibit the growth of *P. aeruginosa* ATCC 15442, *E. coli* ATCC 11220, and *S. enterica* ATCC 14028 bacteria at the concentrations applied. The isolated compounds were tested in vitro only against the *S. aureus* ATCC 29213 strain, since this was the only microorganism susceptive to the SL mixture in the disk diffusion assays. The MIC found for the SL mixture over *S. aureus* was 750 μg mL^{-1}. This result confirms that the SLs possess antimicrobial effect, besides their biological and therapeutic activities [11]. This result is of great importance particularly because *S. aureus* is well known by its resistance toward antibiotics [11, 37].

In the work of Joung et al. [12] the minimum inhibitory concentration values for the *n*-hexane fraction—the only fraction studied in the MIC assays—which was obtained after the methanolic extraction of yacon leaves, ranged from 3.9 to 62.5 μg mL^{-1} in the absence of light, and from 2.0 to 31.3 μg mL^{-1} in its presence. On six of the seven *S. aureus* strains evaluated, the use of light leveraged the inhibition potential of the extract, lowering the MIC needed in about 50% when compared to the assays performed in the dark.

In the present work, the enhydrin and uvedalin mixture did not exert this potentiating effect in the presence of light. Since Joung et al. [12] applied leaf extract as a antimicrobial agent, the improvement found by them in the experiments under the presence of light most likely occurred due to other components than the main SLs of yacon leaves. Another possibility could be the use of a different *S. aureus* strain. However, while the antibiotic-resistant *Staphylococcus* from Joung et al. [12] did not present a higher susceptibility under light, the *Staphylococcus* strain used in this work showed a low-to-moderate resistance to antibiotics. The use of light as a means of improving the antibacterial effect of the extract of yacon leaves should be further investigated using a range of catalogued *Staphylococcus* strains and combinations of SLs.

It is a tough task to consolidate a global perspective of the antimicrobial activity of compounds. This is mainly due to the great amount of microbial types and strains that exist, whether known or not. Cartagena et al. [30], for

instance, verified that uvedalin presented no antimicrobial activity against *S. aureus* ATCC 6538P (the same strain of the present work) and F7 strains, *E. faecalis* ATCC 39212, *Lactobacillus* strains, and Gram-negative strains. Complementarily, Choi et al. [38] verified the antibacterial activity of enhydrin, polymatin B and allo-schkuhriolide (PubChem CID: 10015562) SLs (commonly found in yacon leaves) against two catalogued *S. aureus* strains (ATCC 33591 and ATCC 25923) and fifteen clinical isolates of the species. Enhydrin was able to affect the bacterial activity of all strains tested, with a MIC ranging from 125 to 500 μg mL^{-1}. In this sense, to achieve a more effective inhibition of a microorganism type, the higher MIC found for a strain type must be taken into account. No MIC presented positive results for the polymatin B and allo-schkuhriolide against the strains tested, at least up to 500 μg mL^{-1} (there is no information regarding the maximum SL concentration tested). No other work in the literature was found to verify the single antimicrobial activity of uvedalin and enhydrin.

Taking these results from Cartagena et al. [30] and Choi et al. [38], we cannot suppose the existence of a synergistic effect of the enhydrin and uvedalin against *S. aureus* strains. However, taking into account the enhydrin-to-uvedalin ratio found in our *S. sonchifolius* samples (3.5:1, respectively), from the MIC of 750 μg mL^{-1} there will be approximately 544 μg of enhydrin mL^{-1} and 206 μg of uvedalin mL^{-1}. Assuming that uvedalin has no toxic effect on *S. aureus* ATCC 6538—as Cartagena et al. [30] verified—the concentration of 544 μg of enhydrin mL^{-1} would be close to the values of MIC range verified by Choi et al. [38]. Of course, this is just an assumption that must confirmed by further studies.

According to the yield obtained in SLs (20 mg), the amount of dried yacon leaves needed to achieve the MIC of the 3.5:1 enhydrin/uvedalin mixture against *S. aureus* ATCC 6538 (750 μg mL^{-1}) in any medium would be approximately 5.62 g mL^{-1}, considering a complete extraction. As this concentration would probably be impossible to achieve in regular extraction processes, proper technologies could be used to concentrate the yacon extract to several media, increasing their antimicrobial power.

Conclusion

In the present study, a mixture of two sesquiterpene lactones was extracted and purified from *S. sonchifolius* leaves. The compounds enhydrin and uvedalin were identified in the mixture. The in vitro tests showed that the SLs used at the same proportion naturally found in yacon leaves did not exert antibacterial activity against Gram-negative bacterial *P. aeruginosa*, *E. coli*, and *S. enterica* strains for concentrations up to 90 μg of SLs per disk. However, an inhibitory activity was observed against *S. aureus*, a Gram-positive bacterium. Future works should involve a more complete study examining the effect of enhydrin + uvedalin mixtures on different microbial species, types, and strains. The sesquiterpene lactones characterization and inhibition data obtained in this study will aid as a primary elucidation of the antimicrobial potential of the main SLs found in *S. sonchifolius* leaves.

Acknowledgements The authors gratefully acknowledge the Brazilian agencies CAPES (Coordination for Enhancement of Higher Education Personnel) and CNPq (National Council for Scientific and Technological Development) for the financial support.

Compliance with ethical standards

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

Compliance with ethics requirements This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Grau A, Rea J (1997) Smallanthus sonchifolius (Poepp. & Endl.) H. Robinson. In: Hermann M, Heller J (eds) Andean roots tubers Ahipa, arracacha, maca yacon. Promot. Conserv. use underutilized neglected Crop. 21. Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany/International Plant Genetic Resources Institute, Rome, Italy, pp 199–242
2. Ojansivu I, Ferreira CL, Salminen S (2011) Yacon, a new source of prebiotic oligosaccharides with a history of safe use. Trends Food Sci Technol 22:40–46. doi:10.1016/j.tifs.2010.11.005
3. de Andrade EF, de Souza Leone R, Ellendersen LN, Masson ML (2014) Phenolic profile and antioxidant activity of extracts of leaves and flowers of yacon (*Smallanthus sonchifolius*). Ind Crops Prod 62:499–506. doi:10.1016/j.indcrop.2014.09.025
4. Valentová K, Ulrichová J (2003) *Smallanthus sonchifolius* and *Lepidium meyenii*—prospective Andean crops for the prevention of chronic diseases. Biomed Pap 147:119–130. doi:10.5507/bp.2003.017
5. Oliveira RB, Chagas-Paula DA, Secatto A et al (2013) Topical anti-inflammatory activity of yacon leaf extracts. Braz J Pharmacogn 23:497–505. doi:10.1590/S0102-695X2013005000032
6. Lin F, Hasegawa M, Kodama O (2003) Purification and identification of antimicrobial sesquiterpene lactones from yacon (*Smallanthus sonchifolius*) leaves. Biosci Biotechnol Biochem 67:2154–2159. doi:10.1271/bbb.67.2154
7. Baroni S, da Rocha BA, Oliveira de Melo J et al (2016) Hydroethanolic extract of *Smallanthus sonchifolius* leaves improves hyperglycemia of streptozotocin induced neonatal diabetic rats. Asian Pac J Trop Med 9:432–436. doi:10.1016/j.apjtm.2016.03.033
8. Schmidt TJ (1999) Toxic activities of sesquiterpene lactones: structural and biochemical aspects. Curr Org Chem 3:577–608
9. Martino R, Beer MF, Elso O et al (2015) Sesquiterpene lactones from *Ambrosia* spp. are active against a murine lymphoma cell line by inducing apoptosis and cell cycle arrest. Toxicol Vitr 29:1529–1536. doi:10.1016/j.tiv.2015.06.011

10. De Ford C, Ulloa JL, Catalán CAN et al (2015) The sesquiterpene lactone polymatin B from *Smallanthus sonchifolius* induces different cell death mechanisms in three cancer cell lines. Phytochemistry 117:332–339. doi:10.1016/j.phytochem.2015.06.020
11. Merfort I (2002) Review of the analytical techniques for sesquiterpenes and sesquiterpene lactones. J Chromatogr A 967:115–130. doi:10.1016/S0021-9673(01)01560-6
12. Joung H, Kwon D-Y, Choi J-G et al (2010) Antibacterial and synergistic effects of *Smallanthus sonchifolius* leaf extracts against methicillin-resistant *Staphylococcus aureus* under light intensity. J Nat Med 64:212–215. doi:10.1007/s11418-010-0388-7
13. Inoue A, Tamogami S, Kato H et al (1995) Antifungal melampolides from leaf extracts of *Smallanthus sonchifolius*. Phytochemistry 39:845–848. doi:10.1016/0031-9422(95)00023-Z
14. Schorr K, Merfort I, da Costa FB (2007) A novel dimeric melampolide and further terpenoids from *Smallanthus sonchifolius* (Asteraceae) and the inhibition of the transcription factor NF-kB. Nat Prod Commun 2:367–374
15. Schorr K, Da Costa FB (2003) A proposal for chemical characterization and quality evaluation of botanical raw materials using glandular trichome microsampling of yacón (*Polymnia sonchifolia*, Asteraceae), an Andean medicinal plant. Braz J Pharmacogn 13:1–3. doi:10.1590/S0102-695X2003000300001
16. Genta SB, Cabrera WM, Mercado MI et al (2010) Hypoglycemic activity of leaf organic extracts from *Smallanthus sonchifolius*: constituents of the most active fractions. Chem Biol Interact 185:143–152. doi:10.1016/j.cbi.2010.03.004
17. Barcellona CS, Cabrera WM, Honoré SM et al (2012) Safety assessment of aqueous extract from leaf *Smallanthus sonchifolius* and its main active lactone, *enhydrin*. J Ethnopharmacol 144:362–370. doi:10.1016/j.jep.2012.09.021
18. Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45:493–496
19. CLSI—Clinical and Laboratory Standards Institute (2006) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. CLSI document M7-A7, 7th edn. Clinical and Laboratory Standards Institute, Wayne
20. Herz W, Bhat SV (1973) Maculatin: an isomer of uvedalin epoxide from *Polymnia maculata*. Phytochemistry 12:1737–1740. doi:10.1016/0031-9422(73)80394-2
21. Herz W, Bhat SV (1970) Isolation and structure of two new germacranolides from *Polymnia uvedalia*. J Org Chem 35:2605–2611. doi:10.1021/jo00833a028
22. Hong SS, Lee SA, Han XH et al (2008) Melampolides from the leaves of *Smallanthus sonchifolius* and their inhibitory activity of LPS-induced nitric oxide production. Chem Pharm Bull (Tokyo) 56:199–202. doi:10.1248/cpb.56.199
23. Ali E, Dastidar PPG, Pakrashi SC et al (1972) Studies on Indian medicinal plants—XXVIII. Tetrahedron 28:2285–2298. doi:10.1016/S0040-4020(01)93572-0
24. Mercado MI, Aráoz MVC, Grau A, Catalán CAN (2010) New acyclic diterpenic acids from yacon (*Smallanthus sonchifolius*) leaves. Nat Prod Commun 5:1721–1726
25. Aliyu AB, Moodley B, Chenia H, Koorbanally NA (2015) Sesquiterpene lactones from the aerial parts of *Vernonia blumeoides* growing in Nigeria. Phytochemistry 111:163–168. doi:10.1016/j.phytochem.2014.11.010
26. Wedge DE, Galindo JCG, Macías FA (2000) Fungicidal activity of natural and synthetic sesquiterpene lactone analogs. Phytochemistry 53:747–757. doi:10.1016/S0031-9422(00)00008-X
27. Giesbrecht AM, Davino SC, Nassis CZ et al (1990) Antimicrobial activity of sesquiterpene lactones. Quim Nova 13:312–314
28. Gershenzon J, Croteau R (1991) Terpenoids. In: Rosenthal GA, Berenbaum MR (eds) *Herbivores: their interactions with secondary plant metabolites* chemical participation, vol I Chemical Participation, 2nd edn. Academic, San Diego, pp 165–219
29. Calzada J, Ciccio JF, Echandi G (1980) Antimicrobial activity of the heliangolide chromolaenide and related sesquiterpene lactones. Phytochemistry 19:967–968
30. Cartagena E, Montanaro S, Bardon A (2008) Improvement of the antibacterial activity of sesquiterpene lactones. Rev Latinoam Química 36:43–51
31. Siriwan D, Naruse T, Tamura H (2011) Effect of epoxides and α-methylene-γ-lactone skeleton of sesquiterpenes from yacon (Smallanthus sonchifolius) leaves on caspase-dependent apoptosis and NF-κB inhibition in human cervical cancer cells. Fitoterapia 82:1093–1101. doi:10.1016/j.fitote.2011.07.007
32. Barrero AF, Oltra JE, Álvarez M et al (2000) New sources and antifungal activity of sesquiterpene lactones. Fitoterapia 71:60–64. doi:10.1016/S0367-326X(99)00122-7
33. Becker K, Heilmann C, Peters G (2014) Coagulase-negative staphylococci. Clin Microbiol Rev 27:870–926. doi:10.1128/CMR.00109-13
34. Pak A, Gonçalez E, Felicio JD et al (2006) Inhibitory activity of compounds isolated from *Polymnia sonchifolia* on aflatoxin production by *Aspergillus flavus*. Braz J Microbiol 37:199–203
35. Trombetta D, Castelli F, Sarpietro MG et al (2005) Mechanisms of antibacterial action of three monoterpenes. Antimicrob Agents Chemother 49:2474–2478. doi:10.1128/AAC.49.6.2474
36. Badawy MEI, Abdelgaleil SAM, Suganuma T, Fuji M (2014) Antibacterial and biochemical activity of pseudoguaianolide sesquiterpenes isolated from *Ambrosia maritima* against plant pathogenic bacteria. Plant Prot Sci 50:64–69
37. Zarai Z, Boujelbene E, Ben Salem N et al (2013) Antioxidant and antimicrobial activities of various solvent extracts, piperine and piperic acid from *Piper nigrum*. LWT Food Sci Technol 50:634–641. doi:10.1016/j.lwt.2012.07.036
38. Choi JG, Kang OH, Lee YS et al (2010) Antimicrobial activity of the constituents of *Smallanthus sonchifolius* leaves against methicillin-resistant *Staphylococcus aureus*. Eur Rev Med Pharmacol Sci 14:1005–1009