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Non-edible parts of *Solanum stramoniifolium* Jacq. – a new potent source of bioactive extracts rich in phenolic compounds for functional foods

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Extracts prepared from leaves, roots, and stems of *Solanum stramoniifolium* Jacq. (Solanaceae) in 80% ethanol have been tested for their *in vitro* antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities with an aim to find new sources of substances for functional foods and food additives. The root extract revealed the highest antioxidant activity in all assays exceeding the trolox capacity, and was the only extract that inhibited nitric oxide production in mouse macrophage cells, showing also the capacity to suppress the growth of all tested human tumor cell lines (MCF-7, NCI-H460, HeLa and HepG2). The leaf extract showed the strongest antimicrobial activity inhibiting all tested clinical isolates. To the author's best knowledge it was the first time that all individual parts of this plant were tested for biological activity together with the phenolic compound characterization.

1. Introduction

In recent years, food industry is interested in the application of naturally occurring phytochemical compounds with biological activity to food products to enhance their nutraceutical value, health benefits, safety and shelf-life.[1] Moreover, customer demand for more natural and safer food additives and the growing number of chronic diseases motivate scientists to search for new substances that would meet such expectations.[2]

Plants from tropical regions, such as Trinidad and Tobago, grow in a highly competitive environment and therefore produce large amounts of secondary metabolites for their defense. These edible and medicinal plants, usually rich in polyphenols, are often a good source of new bioactive compounds.[3] *Solanum stramoniifolium* Jacq. (coco-chat) is a hairy fruited pea-eggplant of the Solanaceae family with distribution in Asia, South America, Mesoamerica, and the Caribbean region. It is a perennial shrub, 1 to 2 meters high and about as broad; its stems, branches as well as leaves are sparsely prickly. Fruits are 1–2 cm in diameter, globose, hairy, orange or red when ripe.[4] The ripe fruits are consumed while leaves and roots are used in traditional medicine to treat thrush, cold, venereal diseases, inflammation, asthma, arthritis, liver problems, malaria and cancer.[5–8]

In *S. stramoniifolium* plants originating from Thailand, fruits have been excessively tested, however other plant parts remain unexplored. The antioxidant activity (DPPH and ABTS tests, respectively) of water and methanol extracts was described as weak and explained by the low total phenolic content in the fruits.[9,10] Methanol and ethyl acetate extracts of fruits inhibited Gram-negative bacteria *Escherichia coli* in the disc diffusion test, however the same extracts showed no activity against *Salmonella typhimurium*, *Shigella sonnei*, *Helicobacter pylori*, *Streptococcus pyogenase*, *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus viridians*, and *Enterococci* sp.[11] On the contrary, the water extract of seeds contained small proteins (MW < 14.4 kDa) with significant antimicrobial activity against both Gram-positive and Gram-negative bacteria with *Bacillus subtilis*, *Bacillus licheniformis* and *Pseudomonas aeruginosa* being the most sensitive in the disc diffusion test, and with no inhibition of *E. coli* and *Klebsiella pneumoniae*.[12] The bioactive compounds of this species are, nevertheless, unexplored. The ethanolic extract of roots revealed the presence of alkaloids, flavonoids, tannins, triterpenes and

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saponins in a Brazilian study.[13] The only study on phytochemical compounds of *S. stramoniifolium* from Trinidad and Tobago described the isolation of solamargine, a solasodine glycoalkaloid.[14]

According to the World Health Organization, chronic disorders such as cancer, diabetes and hypertension are becoming the major causes of mortality not only in Trinidad and Tobago, but also worldwide.[15] Therefore, it would be desirable to search for new tropical plant sources rich in bioactive compounds that can be applied either as nutraceuticals or in functional foods to fight and prevent these diseases. The combination of the health benefits, lately required by consumers, and the positive role in food safety and storage due to the strong antimicrobial and antioxidant activity of this plant may be of great interest to the modern food industry in development of new products.

To the author's best knowledge, this is the first detailed study of individual parts, such as leaves, stems and roots of *S. stramoniifolium* reporting their anti-inflammatory, antimicrobial, antioxidant, and cytotoxic activities associated with the phenolic compound profiles.

2. Materials and methods

2.1. Reagents and standards

Acetonitrile 99.9% of HPLC grade was from Fisher Scientific (Lisbon, Portugal). The standards trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), β-carotene and ellipticine were purchased from Sigma-Aldrich (St Louis, MO, USA), as also acetic acid, phosphate buffered saline (PBS), sulforhodamine B (SRB), and lipopolysaccharide (LPS). Phenolic compound standards were from Extrasynthèse (Genay, France). DPPH (2,2-diphenyl-1-picrylhydrazyl) was obtained from Alfa Aesar (Ward Hill, MA, USA). The Griess reagent system was purchased from Promega Corporation (Madison, WI, USA). The culture media Muller Hinton broth (MHB) and Tryptic Soy Broth (TSB) were obtained from Biomerieux (Marcy l'Etoile, France). The dye *p*-iodonitrotetrazolium chloride (INT) was purchased from Sigma-Aldrich (Spruce Street; St Louis, MO) and was used as a microbial growth indicator. All other chemicals were of analytical purity and obtained from common suppliers. Water was treated *via* the purification system Milli-Q water (TGI Pure Water Systems, Greenville, SC, USA).

2.2. Plant material

Plant material was harvested during May 2015 in Santa Cruz area (Trinidad), after consultation with local healers. Table 1 presents the botanical name, local names, plant parts investigated and popular uses of the plant in natural medicine. The samples were authenticated by Dr Walcott at the National Herbarium, University of West Indies, St Augustine Campus, Trinidad and voucher specimen TRIN 40646 was deposited thereby.

2.3. Preparation of plant extracts

Leaves, stems and roots were air dried separately right after harvesting and ground to a fine powder by using an electric laboratory scale mill (Grindomix, Retsch, Germany). Each sample (1.5 g) was extracted twice with 30 mL of ethanol/water (80 : 20, v/v) for 1 hour at 150 rpm and room temperature. Subsequently, the supernatant was filtered through Whatman No. 4 filter paper. Ethanol was then evaporated under vacuum at 40 °C (Büchi R-210; Flawil, Switzerland) and the water residue was lyophilized (FreeZone 4.5 model 7750031, Labconco, Kansas City, MO, USA). The resulting fine powder (20 mesh) was mixed to yield homogenized crude extracts and stored in the dark at room temperature until tested. The methodology routinely used in our laboratory was modified according to ethnopharmaceutical requirements on solvents.[16]

2.4. Phenolic compounds' profile

A routine method used in our laboratory was followed.[17] Dry lyophilized extracts were re-dissolved in water/ethanol (80 : 20, v/v) using a sonic bath, filtered through a 0.22 μm nylon filter and subjected to HPLC analysis.

Chromatographic data were acquired using a Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA). This system consists of a diode array detector coupled to an electrospray ionization mass detector (LC-DAD-ESI/MS^n), a quaternary pump, an auto-sampler (kept at 5 °C), a degasser

Table 1 Ethnomedicinal information on *Solanum stramoniifolium* Jacq

Family	Synonyms	Vernacular names	Ethnomedicinal use
Solanaceae	*Solanum demerarense* Dunal	**Trinidad**: coco-chat; **Brazil**: jóa, jurubeba	Root: toothache, venereal diseases, malaria, fever, cancer[6]
	Solanum hirsutum Herb. Peurari ex Dunal	**Colombia**: e-to-pa-a, kobu-yá, uvilla	**Leaves**: thrush, cold, sores[6]
	Solanum maccai Dunal	**Guyana**: bura bura	**Fruits**: sores, irritations, ant bites[6]
	Solanum platyphyllum Dunal	**Peru**: shiwánkush, coconilla[27]	**Whole plant**: chest pain, asthma,[5] liver problems[7]
	Solanum stramonifolium Jacq.	**India**: ram begun, tide begal[5]	
	Solanum toxicarium Lam.		
	Solanum toxicarum Rich.		
	Solanum trichocarpum Miq.		
	Solanum undecimangulare Willd. ex Roem. & Schult.[27]		

and an automated thermostated column section (kept at 35 °C). The Waters Spherisorb S3 ODS-2 C_{18} (3 μm, 4.6 × 150 mm, Waters, Milford, MA, USA) column was used for chromatographic separations. The solvents used were (A) 0.1% formic acid in water and (B) acetonitrile. The gradient elution applied was: 15% B (0–5 min), 15% B to 20% B (5–10 min), 20–25% B (10–20 min), 25–35% B (20–30 min), 35–50% B (30–40 min), the column was then re-equilibrated, using a flow rate of 0.5 mL min^{-1}. Data were collected simultaneously with a DAD (280 and 370 nm) and in a mass spectrometer. Negative mode was chosen for MS detection on a Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA). Sheath gas (nitrogen) was kept at 50 psi. Other parameter settings: source temperature: 325 °C, spray voltage: 5 kV, capillary voltage: −20 V, tube lens offset: −66 V, collision energy: 35 arbitrary units. The full scan captured the mass between *m*/*z* 100 and 1500. The Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA) was used for data acquisition.

For identification of the phenolic compounds, retention times, UV-VIS and mass spectra were compared with available standards. Data from the literature were used to tentatively identify the remaining compounds. Calibration curves of available phenolic standards were constructed based on the UV signal to perform quantitative analysis. Identified phenolic compounds with unavailable commercial standard were quantified *via* calibration curves of the most similar standard available. The results were expressed as mg g^{-1} of dry extract.

2.5. Biological activity screening

Antibacterial activity. Clinical isolates from patients hospitalized in the Local Health Unit of Bragança and Hospital Centre of Trás-os-Montes and Alto-Douro-Vila Real, Northeast of Portugal were used in the assay. Four Gram-positive bacteria (*Enterococcus faecalis* isolated from urine; *Listeria monocytogenes* isolated from cerebrospinal fluid; MSSA: methicillin-sensitive *Staphylococcus aureus* isolated from wound exudate and MRSA: methicillin-resistant *Staphylococcus aureus*, isolated from expectoration), and six Gram-negative bacteria (*Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolated from expectoration; *Escherichia coli*, *Escherichia coli* spectrum extended producer of β-lactamases (ESBL); *Klebsiella pneumoniae*, *Klebsiella pneumoniae* ESBL, all isolated from urine) were used to screen the antibacterial activity of the extracts. Microorganism identification and susceptibility tests were performed on the MicroScan panels (MicroScan®; Siemens Medical Solutions Diagnostics, West Sacramento, CA, USA) using the microdilution method. The interpretation criteria were based on Interpretive Breakpoints as indicated in the Clinical and Laboratory Standards Institute[18] and in the European Committee on Antimicrobial Susceptibility Testing.[19]

A microdilution method with rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay according to Kuete *et al.*[20] with some modifications was performed. The extract was diluted in appropriate media according to bacterial requirements and successive dilutions were carried out in the wells (20 to 0.156 mg mL^{-1} of final concentration). Three negative controls (MHB/TSB, the extract, and medium with antibiotic) and a positive control (MHB and each inoculum) were prepared. For the Gram-negative bacteria, negative control antibiotics, such as amikacin (*K. pneumoniae* ESBL and *P. aeruginosa*), tobramycin (*A. baumannii*), amoxicillin/clavulanic acid (*E. coli* and *K. pneumoniae*) and gentamicin (*E. coli* ESBL) were used. The concentration used was based on the MIC obtained (Table 2). For the Gram-positive bacteria, ampicillin (*L. monocytogenes*) and vancomycin (MSSA, MRSA and *E. faecalis*) were used (Table 3).

Table 2 Resistance profile of Gram-negative bacteria to different antibiotics; MIC values (μg ml^{-1})

Antibiotics	*A. baumannii*		*E. coli*		*E. coli* ESBL		*K. pneumoniae*		*K. pneumoniae* ESBL		*P. aeruginosa*	
Ampicillin	na		>8	R	na		>8	R	≥32	R	na	
Amoxicillin/clavulanic acid	na		≤8/4	S	na		≤8/4	S	≥32	R	na	
Amikacin	na			na	16	I	na		≤2	S	≤8	S
Cefuroxime	na		≤4	S	na		>8	R	≥64	R	na	
Cefotaxime	>32	R	≤1	S	na		>2	R	≥64	R	na	
Ceftazidime	16	I	≤1	S	≥64	R	na		16	R	>8	R
Norfloxacin	na		>8	R	na		>1	R	na		na	
Levofloxacin	na			na	na		na		≥8	R	>2	R
Ciprofloxacin	>2	R	>1	R	0.5	S	>1	R	≥4	R	>1	R
Nitrofurantoin	na		≤32	S	na		>64	R	256	R	na	
Fosfomycin	na		≤16	S	na		≤32	S	na		na	
Colistin	na			na	≤0.5	S	na		na		≤4	S
Gentamicin	4	R	>4	R	≤1	S	≤2	S	≥16	R	>4	R
Imipenem	na			na	0.5	S	na		na		>8	R
Meropenem	na			na	≤0.25	S	na		≤0.25		>8	R
Piperacillin/tazobactam	na			na	≤4	I	≤8	S	≥128	R	>16	R
Trimethoprim/sulfamethoxazole	na		>4/76	R	≤20	S	>4/76	R	≥320	R	na	
Tobramycin	≤2	S		na	≥16	R	na		≥16	R	>4	R

S – susceptible; I – intermediate; R – resistant; classification according to the interpretative breakpoints suggested by Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST); na – not applicable.

Table 3 Resistance profile of Gram-positive bacteria to different antibiotics; MIC values (μg ml^{-1})

Antibiotics	MRSA		MSSA		*E. faecalis*		*L. monocytogenes*	
Penicillin	>8	R	≤0.12	S	na		na	
Ampicillin	na		na		≤4	S	≤0.2	S
Oxacillin	>0.25	R	≤0.25	S	na		na	
Clindamycin	na		>0.5	R	na		na	
Erythromycin	na		>2	R	na		na	
Ceftaroline	≤1	S	na		na		na	
Gentamicin	na		≤1	S	na		na	
Ciprofloxacin	na		>1	R	na		na	
Levofloxacin	na		>2	R	na		na	
Nitrofurantoin	na		na		≤64	S	na	
Linezolid	≤4	S	na		na		na	
Trimethoprim/sulfamethoxazole	na		≤2/38	S	na		≤2/38	S
Vancomycin	≤2	S	≤2	S	≤2	S	na	

MSSA – methicillin-sensitive *Staphylococcus aureus*; MRSA – methicillin-resistant *Staphylococcus aureus*; S – susceptible; I – intermediate; R – resistant; classification according to the interpretative breakpoints suggested by Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST); na – not applicable.

MIC was defined as the lowest extract concentration that prevented the color change (from yellow dye to dark pink), caused by viable microorganisms, and exhibited the complete inhibition of bacterial growth.

Antioxidant activity. Hydroethanolic extracts were re-dissolved in ethanol/water (80 : 20, v/v) to the final concentration of 20 mg mL^{-1} and further diluted to 0.156 mg mL^{-1} to be subjected to the following assays. The antioxidant activity was evaluated by DPPH radical-scavenging activity, reducing power, inhibition of β-carotene bleaching in the presence of linoleic acid radicals and inhibition of lipid peroxidation using TBARS in brain homogenates.[21] The extract concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene bleaching and TBARS assays) or absorbance at 690 nm (reducing power assay) against extract concentrations. Trolox was used as a positive control.

Anti-inflammatory activity. The method previously described by Correa *et al.*[22] was performed in a concentration range 400–125 μg mL^{-1}. Dexamethasone (50 μM) was used as a positive control. The mouse macrophage-like cell line RAW 264.7 stimulated with LPS was used in the assay. Nitric oxide (NO) production was studied with a Griess Reagent System kit. Results were expressed as EC_{50} values (μg mL^{-1}) equal to the sample concentration providing a 50% inhibition of NO production.

Cytotoxicity. Dry extracts (stock concentration 8 mg mL^{-1}, re-dissolved in water) were further diluted to different concentrations to be subjected to *in vitro* antitumor activity and hepatotoxicity evaluation at final well concentrations (400–1.5 μg mL^{-1}). The cytotoxicity was determined using four human tumour cell lines, HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma) and NCI-H460 (non-small cell lung cancer), following a procedure already described by the authors.[17] The cell growth inhibition was measured using sulforhodamine B assay, where the amount of pigmented cells is directly proportional to the total protein mass and therefore to the number of bounded cells.

For hepatotoxicity evaluation, a freshly harvested porcine liver, obtained from a local slaughter house, was used in order to obtain the cell culture, designated as PLP2. The growth inhibition was evaluated using the SRB assay, as previously described.[23] The results were expressed in GI_{50} values; sample concentration that inhibited 50% of the net cell growth. Ellipticine was used as a positive control.

2.6. Statistical analysis

Three repetitions (or two repetitions in case of antimicrobial assay) of the samples were used and triplicates for each concentration reading were carried out in all the assays. Results are expressed as mean values and standard deviations (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $p = 0.05$. When necessary, a Student's *t*-test was used to determine the significant difference among two different samples, with $p = 0.05$. Both statistical treatments were carried out using the SPSS v. 23.0 program.

3. Results and discussion

3.1 Phenolic compounds' profile

Tables 4 and 5 present chromatographic data and tentative determination of phenolic compounds in the hydroethanolic extracts of leaves, stems, and roots of *Solanum stramoniifolium* Jacq. In leaves, 6 phenolic acid derivatives and 14 flavonoids (flavonol glycoside derivatives) were confirmed. Compounds **2** and **6** were positively identified as protocatechuic acid and 5-*O*-caffeoylquinic acid (chlorogenic acid) after comparing the obtained LC-MS data with those of commercial standards. Compound **5** was tentatively assigned as the corresponding *cis* isomer of 5-*O*-caffeoylquinic acid based on its fragmentation pattern and lower levels compared with peak **6**. Furthermore, *cis* hydroxycinnamoyl derivatives would be expected to elute before the corresponding *trans* ones, as observed after UV irradiation (366 nm, 24 h) of hydroxycinnamic acids in our

Table 4 Retention time (R_t), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, and tentative identification of phenolic compounds in the hydroethanolic extract of *Solanum stramoniifolium* leaves

Compound	R_t (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (m/z)	MS^2 (m/z)	Tentative identification	Quantification (mg g^{-1} dry extract)
1	5.1	328	353	191(100), 179(45), 172(4), 135(56)	3-*O*-Caffeoylquinic acid	6.49 ± 0.05
2	5.7	262, 292sh	153	119(100)	Protocatechuic acid	0.37 ± 0.09
3	6.7	328	353	191(20), 179(19), 173(40), 135(27)	*cis*-4-*O*-Caffeoylquinic acid	1.73 ± 0.13
4	7.2	328	353	191(24), 179(28), 173(60), 134(48)	*trans*-4-*O*-Caffeoylquinic acid	2.59 ± 0.23
5	7.5	328	353	191(100), 179(12), 161(5), 135(20)	*cis*-5-*O*-Caffeoylquinic acid	2.21 ± 0.02
6	8.0	328	353	191(100), 179(52), 161(5), 135(34)	*trans*-5-*O*-Caffeoylquinic acid	3.66 ± 0.05
7	15.2	358	625	463(5), 301(100)	Quercetin-*O*-dihexoside	0.11 ± 0.01
8	15.8	352	755	609(33), 301(100)	Quercetin-*O*-deoxyhexosyl-*O*-rutinoside	2.49 ± 0.01
9	16.6	350	755	593(100), 285(38)	Kaempferol-*O*-hexosyl-*O*-rutinoside	1.67 ± 0.01
10	17.2	354	609	301(100)	Quercetin-3-*O*-rutinoside	0.707 ± 0.004
11	17.6	350	739	593(36), 285(95)	Kaempferol-*O*-deoxyhexosyl-*O*-rutinoside	4.7 ± 0.1
12	17.9	346	755	593(100), 469(50), 285(72)	Kaempferol-*O*-hexosyl-*O*-deoxyhexosyl-hexoside	3.0 ± 0.1
13	18.3	356	769	623(40), 315(100)	Isorhamnetin-*O*-deoxyhexoside-*O*-rutinoside	1.57 ± 0.01
14	19.6	350	593	285(100)	Kaempferol-3-*O*-rutinoside	1.8 ± 0.1
15	20.6	354	623	315(100)	Isorhamnetin-*O*-deoxyhexosyl-hexoside	1.31 ± 0.05
16	23.9	356	623	315(100)	Isorhamnetin-3-*O*-rutinoside	0.366 ± 0.007
17	24.7	350	447	285(100)	Kaempferol-3-*O*-glucoside	0.45 ± 0.03
18	25.4	354	477	315(100)	Isorhamnetin-3-*O*-glucoside	1.5 ± 0.1
19	26.3	300sh, 334	771	609(51), 301(44)	Quercetin-*O*-caffeoyl-rutinoside	0.78 ± 0.02
20	28.3	296sh, 332	755	593(9), 285(61)	Kaempferol-*O*-caffeoyl-rutinoside	1.5 ± 0.1
					Total phenolic acids	17.1 ± 0.5
					Total flavonoids	22.0 ± 0.3
					Total phenolic compounds	39.1 ± 0.7

Table 5 Retention time (R_t), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, and tentative identification of phenolic compounds in the hydroethanolic extract of *Solanum stramoniifolium* roots and stems

Compound	R_t (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (m/z)	MS^2 (m/z)	Tentative identification	Quantification (mg g^{-1} dry extract)		Student's *t*-test
						Roots	Stems	
5	7.3	328	353	191(100), 179(12), 161(5), 135(20)	*cis*-5-*O*-Caffeoylquinic acid	2.62 ± 0.22	1.26 ± 0.01	<0.001
6	7.9	328	353	191(100), 179(52), 161(5), 135(34)	*trans*-5-*O*-Caffeoylquinic acid	5.03 ± 0.14	3.42 ± 0.02	<0.001
21	17.4	236, 296, 320sh	472	350(40), 308(31)	Bis(dihydrocaffeoyl) spermidine isomer 1	1.86 ± 0.16	0.43 ± 0.01	<0.001
22	20.3	226, 294, 322sh	799	637(100), 515(6), 472(10), 350(3), 308(3)	Tris(dihydrocaffeoyl) spermidine hexoside	0.63 ± 0.10	1.17 ± 0.01	<0.001
23	24.3	284	637	515(23), 472(47), 350(15), 308(8)	Tris(dihydrocaffeoyl) spermidine	9.51 ± 0.08	1.06 ± 0.02	<0.001
24	29.4	226, 284, 316sh	472	350(32), 308(38)	Bis(dihydrocaffeoyl) spermidine isomer 2	0.78 ± 0.02	0.46 ± 0.05	<0.001
25	31.1	226, 292, 320sh	472	350(30), 308(48)	Bis(dihydrocaffeoyl) spermidine isomer 3	0.55 ± 0.09	1.08 ± 0.05	<0.001
					Total phenolic compounds and derivatives	20.98 ± 0.81	8.89 ± 0.01	<0.001

laboratory.[24] *cis* and *trans* isomers of 4-*O*-caffeoylquinic acid (compounds **3** and **4**) and *trans* 3-*O*-caffeoylquinic acid (compound **1**) were distinguished and identified by typical fragmentation patterns as described by Clifford *et al.*[25,26] To the best of our knowledge these compounds were described in *Solanum stramoniifolium* Jacq. for the first time.

The flavonol derivatives detected in the leaf extract were mainly glycosides of quercetin (λ_{max} around 354 nm; MS^2 frag-

ment *m*/*z* 301), isorhamnetin (λ_{max} around 356 nm; MS^2 fragment *m*/*z* 317), and kaempferol (λ_{max} around 348 nm, MS^2 fragment *m*/*z* 285).

Quercetin-3-*O*-rutinoside (rutin; compound **10**), kaempferol-3-*O*-rutinoside (nicotiflorin; compound **14**), isorhamnetin-3-*O*-rutinoside (narcissin; compound **16**), kaempferol-3-*O*-glucoside (astragalin; compound **17**) and isorhamnetin-3-*O*-glucoside (compound **18**) were positively identified upon comparison of their retention times, UV-Vis characteristics and mass spectra with available commercial standards.

Compound **7** presented a pseudomolecular ion $[M - H]^-$ at *m*/*z* 625, releasing a MS^2 fragment at *m*/*z* 301 ($[M - H - 162 - 162]^-$, loss of two hexosyl moieties), which led to its tentative identification as quercetin-*O*-dihexoside. Compounds **8**, **11**, and **13** provided the same fragmentation losses of deoxyhexose (146 u) and deoxyhexosyl-hexose (308 u), indicating the location of each residue on different positions of the aglycons of quercetin, kaempferol, and isorhamnetin ($[M - H]^-$ at *m*/*z* 755, 739, and 769, respectively). Similarly, MS^2 fragments of peaks **9** and **12** revealed the alternative loss of hexosyl (*m*/*z* at 593; −162 u) and deoxyhexosyl-hexose (*m*/*z* at 285; −308 u) residues. The positive identification of present rutinosides, including quercetin-3-*O*-rutinoside, in the samples may suggest a rutinoside identity for the deoxyhexosyl-hexose residues in peaks **8**, **9**, **11** and **13**. However, in the case of peak **12**, the information about the identity of the sugar moieties and location onto the aglycon could not be confirmed, therefore the compound was tentatively identified as kaempferol-*O*-hexosyl-*O*-deoxyhexosyl-hexoside. Compound **15** ($[M - H]^-$ at *m*/*z* 623) presented the same pseudomolecular ion as compound **16**, but showed an earlier retention time. The observation of just a single MS^2 fragment (*m*/*z* at 315; −308 u), could indicate that the two sugar units were linked together and the compound was tentatively assigned as isorhamnetin-*O*-deoxyhexosyl-hexoside.

Compounds **19** ($[M - H]^-$ at *m*/*z* 771) and **20** ($[M - H]^-$ at *m*/*z* 755) could correspond to compounds including an acylation with a phenolic acid. The observation in their fragmentation of a product ion at *m*/*z* 609 and 593, respectively, from the losses of caffeoyl residue (162 u), could also be coherent with that identity, as well as the late elution, since the presence of the hydroxycinnamoyl residue implies a decrease in polarity. Therefore, these molecules were tentatively assigned to quercetin-*O*-caffeoyl-rutinoside and kaempferol-*O*-caffeoyl-rutinoside.

The root and stem extracts gave a similar phenolic profile, obtaining different quantities of seven identified compounds. Compounds **5** and **6** were identified as 5-*O*-caffeoylquinic isomers *cis*- and *trans*- as described above. The root extract gave higher amounts of these substances than the stem extract. Compounds **21**, **24**, and **25** ($[M - H]^-$ at *m*/*z* 472) were thought to represent polyamine derivatives, namely three isomers of *N*,*N*′-bis(dihydrocaffeoyl)spermidine as described in the literature by Parr *et al.*[27] Similarly, and taking into account the findings reported by Gancel *et al.*[28] compound **23** ($[M - H]^-$ at *m*/*z* 637) lead to *N*,*N*′,*N*″-tris(dihydrocaffeoyl)spermidine and its hexoside, compound **22**; $[M - H]^-$ at *m*/*z* 799, which gives a MS^2 fragment at *m*/*z* 637 $[M - H - 162]^-$. Nevertheless, a complete identification of the position of dihydrocaffeoyl groups on the spermidine skeleton was not possible. Compound **23** was the most abundant compound present in both parts of this species.

Flavonoids were the most abundant group of phenolic compounds identified in the present study. Nevertheless, polyamine derivatives (spermidines) were dominant in the root and stem extracts. To date, no record exists on spermidine derivatives in *S. stramoniifolium*, however, their presence was frequently described in other representatives of Solanum genus, such as potato (*S. tuberosum*) or naranjilla fruit (*S. quitoense*).[28,29]

3.2. Biological activity

The increasing number of bacterial strains resistant to severe available antibiotics remains a huge problem and is a driving force for the search of new compounds with antimicrobial activity.[30] Furthermore, the food industry calls for natural antimicrobial additives that would be efficient and safe for human consumption at the same time. Various natural peptides, polysaccharides, terpenes, and phenolic compounds have been applied as food preservatives with no toxicity, such as thymol, carvacrol, chitosan, and nisin.[31]

The crude extracts of leaves, stems, and roots of *S. stramoniifolium* were tested for antimicrobial activity against selected clinical isolates representing both Gram-positive and Gram-negative bacteria: *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, all known to exhibit multi-resistance to antibiotics and labeled as the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species).[32] It is established that the Gram-negative bacteria possess stronger resistance due to their protective outer membrane rich in lipopolysaccharides,[33] which is missing in Gram-positive bacteria.

In Table 6, the results obtained from a broth microdilution method with INT colorimetric evaluation are displayed. As can

Table 6 Antibacterial activity of *Solanum stramoniifolium* hydroethanolic extracts (MIC; mg mL^{-1})

	MIC (mg mL^{-1})		
Bacteria	Leaf	Root	Stem
Gram-positive strains			
MRSA	5	10	5
MSSA	5	10	5
Enterococcus faecalis	5	10	10
Listeria monocytogenes	20	10	2.5
Gram-negative strains			
Acinetobacter baumannii	10	10	>20
Escherichia coli	5	10	20
Escherichia coli ESBL	5	10	20
Klebsiella pneumoniae	5	10	20
Klebsiella pneumoniae ESBL	5	10	20
Pseudomonas aeruginosa	10	20	>20

ESBL = spectrum extended producer of β-lactamases. MIC = minimal inhibition concentration. MRSA = methicillin-resistant *Staphylococcus aureus*. MSSA = methicillin-sensitive *Staphylococcus aureus*.

be seen, all three extracts exhibited antimicrobial activity to all the assayed bacteria, and MICs ranged from 2.5 to 20 mg mL^{-1}. In two cases, the MIC was above the maximal tested concentration (stem extract against *A. baumannii* and *P. aeruginosa*). In general, the Gram-positive bacteria were more sensitive to the extracts than Gram-negative bacteria, as expected. However, the root extract presented non-selective inhibition providing the same MIC values for 9 of 10 bacterial strains (10 mg mL^{-1}). On the other hand, the stem extract was significantly more active against Gram-positive bacteria. *Listeria monocytogenes* was the most susceptible organism providing the lowest MICs in stem extract (2.5 mg mL^{-1}). *P. aeruginosa* was the least inhibited organism in the assay. Overall, the leaf extract was the most effective inhibitor with MICs of 5 mg mL^{-1} obtained for 7 clinical isolates. Notably, the bacteria with special characteristics, such as methicillin-resistant MRSA or β-lactamase producing *E. coli* and *K. pneumoniae*, did not present higher MICs than their more sensitive analogues. The water extract of seeds from *S. stramoniifolium* (Thailand) showed significant multispectral inhibition (*S. aureus*, *P. aeruginosa*, B*acillus. subtilis*, *Bacillus licheniformis*, *Xanthomonas* sp., *Salmonella typhi*), however inhibition of *E. coli* and *K. pneumoniae* were not observed in the disc diffusion test.[12]

From the phenolic compounds identified in the plant parts, nicotiflorin, rutin, and chlorogenic acid were previously related with antimicrobial activity in the Solanum genus[34] and therefore can contribute to the inhibitory potential of this species.

The results of antioxidant, anti-inflammatory and cytotoxic activity are included in Table 7, due to their possible relationship previously described in the literature.[35,36] Polyphenol extracts have been used in the food industry as they often exert multiple biological activities in protection against spoilage and oxidation *via* synergism of the compounds they contain.[31]

The antioxidant activity was evaluated using four *in vitro* assays covering various mechanisms, such as hydrogen atom transfer (HAT) and single electron transfer (SET), to fully unfold the antioxidant capacity of the studied samples.[37]

As it can be observed in Table 7, all plant part extracts showed significant antioxidant potential in the four assays (DPPH; reducing power, β-carotene bleaching inhibition and TBARS). The root extract stands out when compared to the other plant parts. It was significantly more effective than trolox standard in all antioxidant assays, providing lower EC_{50} values in each of the tested assays. Regarding DPPH scavenging capacity assay, the plant parts were declining as follows: root > leaf > stem with the corresponding EC_{50} values of 13 ± 1; 50 ± 2 and 74 ± 4 μg mL^{-1}, respectively. In reducing power assay, two extracts provided better results than the standard trolox (EC_{50} = 41.7 ± 0.3 μg mL^{-1}), namely root and leaf (EC_{50} of 8.68 ± 0.03 and 23.7 ± 0.1 μg mL^{-1}, respectively). The order of activity in reducing power was: root > leaf > stem, as observed in DPPH assay as well. Moreover, the same two extracts proved to be better β-carotene bleaching inhibitors than trolox, as only the stem extract gave a higher EC_{50} value than this standard (23.4 ± 0.4 *versus* 18 ± 1 μg mL^{-1}). In the TBARS inhibition test, only the root extract exceeded trolox capacity, however the results were still quite promising (root > leaf > stem; EC_{50} values corresponding to 15 ± 1; 33 ± 1 and 60 ± 1 μg mL^{-1}, respectively). Previously, Wetwitayaklung and Phaechamud[10] observed low scavenging activity for the methanol fruit extract of *S. stramoniifolium* in TEAC assay using the $ABTS^{+\bullet}$ radical (IC_{50} = 1133.08 μg compared to 10.14 μg for trolox) and correlated it to the low presence of total phenolic compounds (1.55 g gallic acid equivalents per 100 g extract).

Table 7 Biological activity of hydroethanolic extracts from different parts of *Solanum stramoniifolium* Jacq

	Leaf	Root	Stem	Trolox
Antioxidant activity (EC_{50} values, μg mL^{-1})				
DPPH scavenging activity	50 ± 2b	13 ± 1d	74 ± 4a	41 ± 1c
β-Carotene bleaching inhibition	11.7 ± 0.1c	9.4 ± 0.5d	24.3 ± 0.4a	18 ± 1b
Reducing power	23.7 ± 0.1c	8.68 ± 0.03d	45 ± 0.3a	41.7 ± 0.3b
TBARS inhibition	33 ± 1b	15 ± 1d	60 ± 1a	23 ± 1c
	Leaf	**Root**	**Stem**	**Dexamethasone**
Anti-inflammatory activity (EC_{50} values, μg mL^{-1})				
Nitric oxide (NO) production	>400	100 ± 6	>400	16 ± 1
	Leaf	**Root**	**Stem**	**Ellipticine**
Cytotoxicity to tumor cell lines (GI_{50} values, μg mL^{-1})				
HeLa (cervical carcinoma)	97 ± 4b	206 ± 15a	>400	1.91 ± 0.06c
HepG2 (hepatocellular carcinoma)	85 ± 6a	40 ± 3b	>400	1.1 ± 0.2c
MCF-7 (breast carcinoma)	206 ± 10b	52 ± 5c	242 ± 4a	0.91 ± 0.04d
NCI-H460 (non-small cell lung cancer)	155 ± 13a	113 ± 5b	>400	1.0 ± 0.1c
Cytotoxicity to non-tumor cell lines (GI_{50} values, μg mL^{-1})				
PLP2 (porcine liver primary culture)	>400	252 ± 10	>400	3.2 ± 0.7

Trolox, dexamethasone and ellipticine, respectively, were used as positive controls in the assays. All values are means ± SD (n = 9) and in each row different letters represent significant differences ($p < 0.05$).

Lipid peroxidation products (*e.g.* malondialdehyde), as well as free radicals, may damage important cell macromolecules, such as DNA, proteins, and lipids and contribute to the development of pathological processes, including aging, cancer, atherosclerosis, coronary heart disease or neurodegenerative problems.[38] Despite the effectiveness of endogenous antioxidant systems, an exogenous source of antioxidants is necessary in the case of excessive presence of oxidative species. Therefore, prevention or limitation of oxidative stress might be achieved by dietary antioxidants, such as phenolic-rich plant extracts.

From the tested plant parts, only the root revealed activity in the NO production (EC_{50} = 100 ± 6 μg mL^{-1}) as stated in Table 7. Leaf and stem did not show any activity within the maximal concentration tested (400 μg mL^{-1}), which is surprising according to the traditional choice of leaves for external inflammation. It can be suggested that other than NO production-related mechanisms are involved and different assays shall be evaluated in future to study this activity.

More than 60% of agents used in cancer therapy are from natural sources, especially tropical plants.[39] The Solanum genus is a good source for anticancer substances, such as solanine or solamargine.[40,41] The antitumor potential was evaluated against four human tumor cell lines represented by MCF-7 (breast carcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma), and porcine liver primary culture PLP2 was selected for cytotoxicity assessment against non-tumor cells. Observing the results presented in Table 7, it can be concluded that leaf and root are the most promising plant parts with antitumor compounds as they inhibited all tumor cell lines used in the study. The highest inhibition was found for HepG2, yielding the lowest GI_{50} (40 ± 3 μg mL^{-1} for root and 85 ± 6 μg mL^{-1} for leaf extract). The stem extract was efficient only in MCF-7 cell line inhibition (GI_{50} = 242 ± 4 μg mL^{-1}). The most sensitive cell line was MCF-7, which was inhibited by all three extracts in the following order root > leaf > stem. Interestingly, the root extract provided lower GI_{50} for HepG2, MCF-7 and NCI-H460 than leaf, but was less effective against HeLa cell line. Compared to ellipticine, the extracts revealed medium activity. Nevertheless, ellipticine has a very strong inhibiting power on all presented tumor cell lines, but also exhibits high hepatotoxicity to non-tumor PLP2 cell line. In our case, only root showed mild hepatotoxicity towards PLP2 (GI_{50} = 252 ± 10 μg mL^{-1}), however it did not exceed active concentrations against the tumor cell lines (40 ± 3 μg mL^{-1} in HepG2; 52 ± 5 μg mL^{-1} in MCF-7; 113 ± 5 μg mL^{-1} in NCI-H460; and 206 ± 15 μg mL^{-1} in HeLa).

Consequently, although the leaf and root extracts of *S. stramoniifolium* could be useful in the development of new anticancer products, the leaf is the most promising part, since it did not present unspecific toxicity, as suggested by results obtained with the PLP2 assay.

Due to the possible synergetic effect of present compounds, the plant crude extracts can often be a more powerful antioxidant tool than individual substances. Moreover, the natural matrices in the form of crude extracts possess usually very low toxicity compared to individual chemicals and therefore are currently experiencing a renaissance in both the phytopharmacological and food industry.[31]

4. Conclusions

This study highlights the potential of different parts of *Solanum stramoniifolium* Jacq. as a rich source of biologically active compounds suitable for applications in the food industry, for example in the development of novel functional foods and nutraceutical formulations. Ethanol/water extracts from leaves, stems, and roots demonstrated to have a strong biological activity. The root extract gave the highest antioxidant potential exceeding trolox standard values. It also significantly inhibited the growth of MCF-7 and HepG2 tumor cell lines. The leaf extract showed the best results in the antimicrobial assay inhibiting all the clinical bacterial isolates. Furthermore, it did not possess any cytotoxicity, unlike the root extract, and therefore might be a better candidate for the food industry. The phenolic compounds in the extracts revealed the content of compounds known for their biological activities, such as caffeoylquinic acid derivatives, flavonoids and polyamines. The presence of these compounds could be correlated with the high biological activity shown by these extracts. Several compounds were determined for the first time in this plant.

Conflict of interest

No conflict of interest.

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