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

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21 **Abstract**

22 Extracts prepared from leaves, roots, and stems of *Solanum stramoniifolium* Jacq. (Solanaceae) in
23 80% ethanol have been tested for their *in vitro* antioxidant, anti-inflammatory, antimicrobial, and
24 cytotoxic activities in aim to find new sources of substances for functional foods and food
25 additives. The root extract revealed the highest antioxidant activity in all assays exceeding the
26 trolox capacity, and was the only extract that inhibited the nitric oxide production in mouse
27 macrophage cells, showing also the capacity to suppress the growth of all tested human tumor
28 cell lines (MCF-7, NCI-H460, HeLa and HepG2). The leaf extract showed the strongest
29 antimicrobial activity inhibiting all tested clinical isolates. To the author's best knowledge it was
30 the first time that all individual parts of this plant were tested for biological activity together with
31 the phenolic compounds characterization.

32

33 *Keywords:* Antioxidant; Anti-inflammatory; Cytotoxicity; Antimicrobial activity; Phenolic
34 compounds; *Solanum stramoniifolium*;

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36 **1. Introduction**

37 Recently, food industry is interested in the application of naturally occurring phytochemical
38 compounds with biological activity into food products to enhance their nutraceutical value, health
39 benefits, safety and shelf-life.[1] Moreover, customers demand for more natural and safer food
40 additives and the growing number of chronic diseases motivates scientist to search for new
41 substances that would meet such expectations.[2]

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

51 In *S. stramoniifolium* plants originating from Thailand, fruits have been excessively tested,
52 however other plant parts remain unexplored. The antioxidant activity (DPPH and ABTS tests,
53 respectively) of water and methanol extracts was described as weak and explained by the low
54 total phenolic content in the fruits.[9,10] Methanol and ethyl acetate extracts of fruits inhibited
55 Gram-negative bacteria *Escherichia coli* in disc diffusion test, however the same extracts showed
56 no activity against *Salmonella typhimurium, Shigella sonnei, Helicobacter pylori*, *Streptococcus*
57 *pyogenase, Salmonella typhi, Staphylococcus aureus, Streptococcus viridians,* and *Enterococci*
58 sp.[11] On the contrary, the water extract of seeds contained small proteins (MW < 14.4 kDa) with

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59 significant antimicrobial activity against both Gram-positive and Gram-negative bacteria with
60 *Bacillus subtilis, Bacillus licheniformis* and *Pseudomonas aeruginosa* being the most sensitive in
61 the disc diffusion test, and with no inhibition of *E. coli* and *Klebsiella pneumoniae*.[12] The
62 bioactive compounds of this species are, nevertheless, an unexplored field. The ethanolic extract
63 of roots revealed the presence of alkaloids, flavonoids, tannins, triterpenes and saponins in a
64 Brazilian study.[13] The only study on phytochemical compounds of *S. stramoniifolium* from
65 Trinidad and Tobago described the isolation of solamargine, a solasodine glycoalkaloid.[14]

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

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

77

78 **2. Materials and methods**

79 **2.1. Reagents and standards**

80 Acetonitrile 99.9% of HPLC grade was from Fisher Scientific (Lisbon, Portugal). The standards
81 trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), β-carotene and ellipticine were

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82 purchased from Sigma-Aldrich (St. Louis, MO, USA), as also acetic acid, phosphate buffered
83 saline (PBS), sulforhodamine B (SRB), and lipopolysaccharide (LPS). Phenolic compound
84 standards were from Extrasynthèse (Genay, France). DPPH (2,2-diphenyl-1-picrylhydrazyl) was
85 obtained from Alfa Aesar (Ward Hill, MA, USA). The Griess reagent system was purchased from
86 Promega Corporation (Madison, WI, USA). The culture media Muller Hinton broth (MHB) and
87 Tryptic Soy Broth (TSB) were obtained from Biomerieux (Marcy l'Etoile, France). The dye p-
88 iodonitrotetrazolium chloride (INT) was purchased from Sigma-Aldrich (Spruce Street; St. Louis,
89 MO) and was used as microbial growth indicator. All other chemicals were of analytical purity
90 and obtained from common suppliers. Water was treated via the purification system Milli-Q
91 water (TGI Pure Water Systems, Greenville, SC, USA).

92 **2.2. Plant material**

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

98

99 **2.3. Preparation of plant extracts**

100 Leaves, stems and roots were air dried separately right after harvesting and grinded to a fine
101 powder by using an electric laboratory scale mill (Grindomix, Retsch, Germany). Each sample
102 (1.5 g) was extracted twice with 30 mL of ethanol/water (80:20, v/v) for 1 hour at 150 rpm and
103 room temperature. Subsequently, the supernatant was filtered through Whatman No. 4 filter
104 paper. Ethanol was then evaporated under vacuum at 40 °C (Büchi R-210; Flawil, Switzerland)

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105 and the water residue was lyophilized (FreeZone 4.5 model 7750031, Labconco, Kansas City,
106 MO, USA). The resulting fine powder (20 mesh) was mixed to yield homogenized crude extracts
107 and stored in the dark at room temperature until tested. The methodology routinely used in our
108 laboratory was modified according to ethnopharmaceutical requirements on solvents.[16]

109

110 **2.4. Phenolic compounds profile**

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

114 Chromatographic data were acquired from Dionex Ultimate 3000 UPLC (Thermo Scientific, San
115 Jose, CA, USA). This system consists of a diode array detector coupled to an electrospray
116 ionization mass detector (LC-DAD-ESI/MSn), a quaternary pump, an auto-sampler (kept at 5
117 ºC), a degasser and an automated thermostatted column section (kept at 35ºC). Waters Spherisorb
118 S3 ODS-2 C_{18} (3 µm, 4.6 × 150 mm, Waters, Milford, MA, USA) column provided
119 chromatographic separations. The solvents used were (A) 0.1% formic acid in water and (B)
120 acetonitrile. The gradient elution applied was: 15% B (0-5 min), 15% B to 20% B (5-10 min), 20-
121 25% B (10-20 min), 25-35% B (20-30 min), 35-50% B (30-40 min), the column was then re-
122 equilibrated, using a flow rate of 0.5 mL/min. Data were collected simultaneously with a DAD
123 (280 and 370 nm) and in a mass spectrometer. Negative mode was chosen for MS detection on a
124 Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA). Sheath gas
125 (nitrogen) was kept on 50 psi. Other parameters settings: source temperature 325 °C, spray
126 voltage 5 kV, capillary voltage -20 V, tube lens offset -66 V, collision energy 35 arbitrary units.

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127 The full scan captured the mass between *m*/*z* 100 and 1500. Xcalibur® data system
128 (ThermoFinnigan, San Jose, CA, USA) was operating the data acquisition.
129 For identification of the phenolic compounds, retention times, UV-VIS and mass spectra were
130 compared with available standards. Data from literature were used to tentatively identify the
131 remaining compounds. Calibration curves of available phenolic standards were constructed based
132 on the UV signal to perform quantitative analysis. Identified phenolic compounds with
133 unavailable commercial standard were quantified via calibration curve of the most similar
134 standard available. The results were expressed as mg/g of dry extract.
135

136 **2.5. Biological activity screening**

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

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151 Microdilution method with rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay
152 according to Kuete *et al*.[20] with some modifications was performed. The extract was diluted in
153 appropriate media according to bacteria requirements and successive dilutions were carried out in
154 the wells (20 to 0.156 mg/mL of final concentration). Three negative controls (MHB/TSB, the
155 extract, and medium with antibiotic) and a positive control (MHB and each inoculum) were
156 prepared. For the Gram-negative bacteria, negative control antibiotics, such as amikacin (*K.*
157 *pneumoniae ESBL* and *P. aeruginosa*), tobramycin (*A. baumannii*), amoxicillin/clavulanic acid
158 (*E. coli* and *K. pneumoniae*) and gentamicin (*E. coli* ESBL) were used. The concentration used
159 was based on the MIC obtained (**Table 2**). For the Gram-positive bacteria, ampicillin (*L.*
160 *monocytogenes)* and vancomycin (MSSA, MRSA and *E. faecalis*) were used (**Table 3**).
161 MIC was defined as the lowest extract concentration that prevented the color change (from
162 yellow dye to dark pink), caused by the viable microorganisms, and exhibited the complete
163 inhibition of bacterial growth.
164 *Antioxidant activity***.** Hydroethanolic extracts were re-dissolved in ethanol/water (80:20, v/v) to
165 the final concentration 20 mg/mL and further diluted to 0.156 mg/mL to be submitted to the
166 following assays. The antioxidant activity was evaluated by DPPH radical-scavenging activity,
167 reducing power, inhibition of β-carotene bleaching in the presence of linoleic acid radicals and
168 inhibition of lipid peroxidation using TBARS in brain homogenates.[21] The extract concentrations
169 providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the
170 graphs of antioxidant activity percentages (DPPH, β-carotene bleaching and TBARS assays) or
171 absorbance at 690 nm (reducing power assay) against extract concentrations. Trolox was used as
172 positive control.

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173 *Anti-inflammatory activity*. Method previously described by Correa *et al.*[22] was performed in
174 concentration range 400 – 125 µg/mL. Dexamethasone (50 µM) was used as a positive control.
175 The mouse macrophage-like cell line RAW 264.7 stimulated with LPS was used in the assay.
176 Nitric oxide (NO) production was studied with Griess Reagent System kit. Results were
177 expressed as EC_{50} values (µg/mL) equal to the sample concentration providing a 50% inhibition
178 of NO production.
179 *Cytotoxicity.* Dry extracts (stock concentration 8 mg/mL, re-dissolved in water) were further
180 diluted to different concentrations to be submitted to *in vitro* antitumor activity and
181 hepatotoxicity evaluation at final well concentrations (400 – 1.5 µg/mL). The cytotoxicity was
182 determined using four human tumour cell lines, HeLa (cervical carcinoma), HepG2
183 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma) and NCI-H460 (non-small cell lung
184 cancer), following a procedure already descried by the authors.[17] The cell growth inhibition was
185 measured using sulforhodamine B assay, were the amount of pigmented cells is directly
186 proportional to the total protein mass and therefore to the number of bounded cells.
187 For hepatotoxicity evaluation, a freshly harvested porcine liver, obtained from a local slaughter
188 house, was used in order to obtain the cell culture, designated as PLP2. The growth inhibition
189 was evaluated using the SRB assay, as previously described.[23] The results were expressed in GI_{50}
190 values; sample concentration that inhibited 50% of the net cell growth. Ellipticine was used as
191 positive control.
192
193 **2.6. Statistical analysis**
194 Three repetitions (or two repetitions in case of antimicrobial assay) of the samples were used and
195 triplicates for each concentration reading were carried out in all the assays. Results are expressed
196 as mean values and standard deviations (SD). The results were analyzed using one-way analysis

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197 of variance (ANOVA) followed by Tukey's HSD test with $p = 0.05$. When necessary, a Student´s
198 *t*-test was used to determine the significant difference among two different samples, with $p =$
199 0.05. Both statistical treatments were carried out using SPSS v. 23.0 program.

200

201 **3. Results and discussion**

202 **3.1 Phenolic compounds profile**

203 **Tables 4A** and **4B** present chromatographic data and tentative determination of phenolic
204 compounds in the hydroethanolic extracts of leaves, stems, and roots of *Solanum stramoniifolium*
205 Jacq. In leaves, 6 phenolic acid derivatives and 14 flavonoids (flavonol glycoside derivatives)
206 were confirmed. Compounds **2** and **6** were positively identified as protocatechuic acid and 5-*O*-
207 caffeoylquinic acid (chlorogenic acid) after comparing the obtained LC-MS data with those of
208 commercial standards. Compound **5** was tentatively assigned as the corresponding *cis* isomer of
209 5-*O*-caffeoylquinic acid based on its fragmentation pattern and lower levels compared with peak
210 **6**. Furthermore, *cis* hydroxycinnamoyl derivatives would be expected to elute before the
211 corresponding *trans* ones, as observed after UV irradiation (366 nm, 24 h) of hydroxycinnamic
212 acids in our laboratory.[24] *Cis* and *trans* isomers of 4-*O*-caffeoylquinic acid (compounds **3** and **4**)
213 and *trans* 3-*O*-caffeoylquinic acid (compound **1**) were distinguished and identified by typical
214 fragmentation patterns as described by Clifford *et al*.[25,26] To the best of our knowledge these
215 compounds were described in *Solanum stramoniifolium* Jacq. for the first time.

216 The flavonol derivatives detected in the leaf extract were mainly glycosides of quercetin (λ_{max}
217 around 354 nm; MS^2 fragment *m/z* 301), isorhamnetin (λ_{max} around 356 nm; MS^2 fragment *m/z*
218 317), and kaempferol (λ_{max} around 348 nm, MS^2 fragment *m/z* 285).

219 Quercetin-3-*O*-rutinoside (rutin; compound **10**), kaempferol-3-*O*-rutinoside (nicotiflorin;
220 compound **14**), isorhamnetin-3-*O*-rutinoside (narcissin; compound **16**), kaempferol-3-*O*-

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221 glucoside (astragalin; compound **17**) and isorhamnetin-3-*O*-glucoside (compound **18**) were
222 positively identified upon comparison of their retention times, UV-Vis characteristics and mass
223 spectra with available commercial standards.
224 Compound **7** presented a pseudomolecular ion $[M-H]^-$ at *m/z* 625, releasing a MS^2 fragment at
225 *m/z* 301 ($[M-H-162-162]^-$, loss of two hexosyl moieties), which led to its tentative identification
226 as quercetin-*O*-dihexoside. Compounds **8**, **11**, and **13** provided the same fragmentation losses of
227 deoxyhexose (146 u) and deoxyhexosyl-hexose (308 u), indicating location of each residue on
228 different positions of the aglycons of quercetin, kaempferol, and isorhamnetin ($[M-H]^-$ at *m/z*
229 755, 739, and 769, respectively). Similarly, MS^2 fragments of peaks **9** and **12** revealed the
230 alternative loss of hexosyl (*m/z* at 593; -162 u) and deoxyhexosyl-hexose (*m/z* at 285; -308 u)
231 residues. The positive identification of present rutinosides, including quercetin-3-*O*-rutinoside, in
232 the samples may suggest a rutinoside identity for the deoxyhexosyl-hexose residues in peaks **8**, **9**,
233 **11** and **13**. However, in case of peak **12**, the information about the identity of the sugar moieties
234 and location onto the aglycon could not be confirmed, therefore the compound was tentatively
235 identified as kaempferol-*O*-hexosyl-*O*-deoxyhexosyl-hexoside. Compound **15** ($[M-H]^-$ at *m/z*
236 623) presented the same pseudomolecular ion as compound **16**, but showed an earlier retention
237 time. The observation of just a single MS^2 fragment (*m/z* at 315; - 308 u), could indicate that the
238 two sugar units were linked together and the compound was tentatively assigned as isorhamnetin-
239 *O*-deoxyhexosyl-hexoside.
240 Compounds **19** **(**$[M-H]^-$ at *m/z* 771) and **20** **(**$[M-H]^-$ at *m/z* 755) could correspond to compounds
241 including an acylation with a phenolic acid. The observation in their fragmentation of a product
242 ion at *m/z* 609 and 593, respectively, from the losses of caffeoyl residue (162 u), could also be
243 coherent with that identity, as well as the late elution, since the presence of the

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244 hydroxycinnamoyl residue implies a decrease in polarity. Therefore, these molecules were
245 tentatively assigned to quercetin-*O*-caffeoyl-rutinoside and kaempferol-*O*-caffeoyl-rutinoside.
246 The root and stem extracts gave a similar phenolic profile, obtaining different quantity of seven
247 identified compounds. Compounds **5** and **6** were identified as 5-*O*-caffeoylquinic isomers *cis*-
248 and *trans*- as described above. The root extract gave higher amounts of these substances than the
249 stem extract. Compounds **21**, **24**, and **25** ($[M-H]^-$ at *m/z* 472) were thought to represent polyamine
250 derivatives, namely three isomers of *N,N′*-bis(dihydrocaffeoyl)spermidine as described in
251 literature by Parr *et al*.[27] Similarly, and taking into account the findings reported by Gancel *et*
252 *al.*[28], compound **23** ($[M-H]^-$ at *m/z* 637) lead to *N,N′,N″*-tris(dihydrocaffeoyl)spermidine and its
253 hexoside, compound **22**; $[M-H]^-$ at *m/z* 799, which gives a MS^2 fragment at *m/z* 637 $[M-H-162]^-$.
254 Nevertheless, a complete identification of the position of dihydrocaffeoyl groups on spermidine
255 skeleton was not possible. Compound **23** was the most abundant compound present in both parts
256 of this species.
257 Flavonoids were the most abundant group of phenolic compounds identified in the present study.
258 Nevertheless, polyamine derivatives (spermidines) were dominant in the root and stem extracts.
259 Up to date, no record exists on spermidine derivatives in *S. stramoniifolium*, however, their
260 presence was frequently described in other representatives of Solanum genus, such as potato (*S.*
261 *tuberosum*) or naranjilla fruit (*S. quitoense*).[28,29]
262

263 **3.2. Biological activity**

264 The increasing number of bacterial strains resistant to severe available antibiotics remains a huge
265 problem and is a driving force for search of new compounds with antimicrobial activity.[30]
266 Furthermore, the food industry calls for natural antimicrobial additives that would be efficient
267 and safe for human consumption at the same time. Various natural peptides, polysaccharides,

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268 terpenes, and phenolic compounds have been applied as food preservatives with no toxicity, such
269 as thymol, carvacrol, chitosan, and nisin.[31]
270 The crude extracts of leaves, stems, and roots of *S. stramoniifolium* were tested for antimicrobial
271 activity against selected clinical isolates representing both Gram-positive and Gram-negative
272 bacteria: *Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa* and
273 *Staphylococcus aureus*, all known to exhibit multi-resistance to antibiotics and labeled as the
274 ESKAPE pathogens (***E****nterococcus faecium*, ***S****taphylococcus aureus*, ***K****lebsiella pneumoniae*,
275 ***A****cinetobacter baumannii*, ***P****seudomonas aeruginosa*, and ***E****nterobacter* species).[32] It is
276 established that the Gram-negative bacteria possess stronger resistance due to their protective
277 outer membrane rich in lipopolysaccharides[33], which is missing in Gram-positive bacteria.
278 In **Table 5**, the results obtained from broth microdilution method with INT colorimetric
279 evaluation are displayed. As it can be seen, all three extracts exhibited antimicrobial activity to all
280 the assayed bacteria, and MICs ranged from 2.5 to 20 mg/mL. In two cases, the MIC was above
281 the maximal tested concentration (stem extract against *A. baumannii* and *P. aeruginosa*). In
282 general, the Gram-positive bacteria were more sensitive to the extracts than Gram-negative
283 bacteria, as expected. However, the root extract presented non-selective inhibition providing the
284 same MIC values for 9 of 10 bacterial strains (10 mg/mL). On the other hand, the stem extract
285 was significantly more active against Gram-positive bacteria. *Listeria monocytogenes* was the
286 most susceptible organism providing the lowest MICs in stem extract (2.5 mg/mL). *P.*
287 *aeruginosa* was the least inhibited organism in the assay. Overall, the leaf extract was the most
288 effective inhibitor with MICs of 5 mg/mL obtained for 7 clinical isolates. Notably, the bacteria
289 with special characteristic, such as methicillin-resistant MRSA or β-lactamase producing *E. coli*
290 and *K. pneumoniae*, did not present higher MICs than their more sensitive analogues. The water
291 extract of seeds from *S. stramoniifolium* (Thailand) showed significant multispectral inhibition

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292 (*S. aureus*, *P. aeruginosa*, B*acillus. subtilis*, *Bacillus licheniformis*, *Xanthomonas* sp., *Salmonella*
293 *typhi*), however inhibition of *E.coli* and *K. pneumoniae* were not observed in the disc diffusion
294 test.[12]
295 From the phenolic compounds identified in the plant parts, nicotiflorin, rutin, and chlorogenic
296 acid were previously related with antimicrobial activity in the Solanum genus [34] and therefore can
297 contribute to the inhibitory potential of this species.
298 The results of antioxidant, anti-inflammatory and cytotoxic activity are included in **Table 6**, due
299 to their possible relationship previously described in literature.[35,36] Polyphenol extracts have been
300 used in food industry as they often exert multiple biological activities in protection against
301 spoilage and oxidation via synergism of the compounds they contain.[31]
302 The antioxidant activity was evaluated using four *in vitro* assays covering various mechanisms,
303 such as hydrogen atom transfer (HAT) and single electron transfer (SET), to fully unfold the
304 antioxidant capacity of studied samples.[37]
305 As it can be observed in **Table 6**, all plant parts extracts showed significant antioxidant potential
306 in the four assays (DPPH; reducing power, β-carotene bleaching inhibition and TBARS). The
307 root extract stands out when compared to the other plant parts. It was significantly more effective
308 than trolox standard in all antioxidant assays, providing lower EC_{50} values in each of the tested
309 assays. Regarding DPPH scavenging capacity assay, the plant parts were declining as follows:
310 root > leaf > stem with corresponding EC_{50} values of 13 ± 1; 50 ± 2 and 74 ± 4 μg/mL,
311 respectively. In reducing power assay, two extracts provided better results than the standard
312 trolox (EC_{50} = 41.7 ± 0.3 μg/mL), namely root and leaf (EC_{50} of 8.68 ± 0.03 and 23.7 ± 0.1
313 μg/mL, respectively). The order of activity in reducing power was: root > leaf > stem, as
314 observed in DPPH assay as well. Moreover, the same two extracts proved to be better β-carotene
315 bleaching inhibitors than trolox, as only the stem extract gave a higher EC_{50} value than this

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316 standard (23.4 ± 0.4 versus 18 ± 1 μg/mL). In TBARS inhibition test, only the root extract
317 exceeded trolox capacity, however the results were still quite promising (root > leaf > stem; EC_{50}
318 values corresponding to 15 ± 1; 33 ± 1 and 60 ± 1 μg/mL, respectively). Previously,
319 Wetwitayaklung and Phaechamud[10] observed low scavenging activity for methanol fruit extract
320 of *S. stramoniifolium* in TEAC assay using $ABTS^{\cdot+}$ radical (IC_{50} = 1133.08 μg comparing to
321 10.14 μg for trolox) and correlated it to the low presence of total phenolic compounds (1.55 g
322 gallic acid equivalents/100 g extract).
323 Lipid peroxidation products (f. e. malondialdehyde), as well as free radicals, may damage
324 important cell macromolecules, such as DNA, proteins, and lipids and contribute to the
325 development of pathological processes, including aging, cancer, atherosclerosis, coronary heart
326 disease or neurodegenerative problems.[38] Despite the effectiveness of endogenous antioxidant
327 systems, an exogenous source of antioxidants is necessary in case of excessive presence of
328 oxidative species. Therefore, prevention or limitation of oxidative stress might be achieved by
329 dietary antioxidants, such as phenolic-rich plant extracts.
330 From the tested plant parts, only root revealed activity in the NO production (EC_{50} = 100 ± 6
331 μg/mL) as stated in **Table 6**. Leaf and stem did not show any activity within the maximal
332 concentration tested (400 μg/mL), which is surprising according to the traditional choice of
333 leaves for external inflammations. It can be suggested that other than NO production-related
334 mechanisms are involved and different assays shall be evaluated in future to study this activity.
335 More than 60% of agents used in cancer therapy are from natural sources, especially tropical
336 plants.[39] The Solanum genus is a good source for anticancer substances, such as solanine or
337 solamargine.[40,41] The antitumor potential was evaluated against four human tumor cell lines
338 represented by MCF-7 (breast carcinoma), NCI-H460 (non-small cell lung cancer), HeLa
339 (cervical carcinoma) and HepG2 (hepatocellular carcinoma), and porcine liver primary culture

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340 PLP2 was selected for cytotoxicity assessment against non-tumor cells. Observing the results
341 presented in **Table 6**, it can be concluded that leaf and root are the most promising plant parts
342 with antitumor compounds as they inhibited all tumor cell lines used in the study. The highest
343 inhibition was found for HepG2, yielding the lowest GI_{50} (40 ± 3 μg/mL for root and 85 ± 6
344 μg/mL for leaf extract). The stem extract was efficient only in MCF-7 cell line inhibition (GI_{50} =
345 242 ± 4 μg/mL). The most sensitive cell line was MCF-7, which was inhibited by all three
346 extracts in the following order root > leaf > stem. Interestingly, the root extract provided lower
347 GI_{50} for HepG2, MCF-7 and NCI-H460 than leaf, but was less effective against HeLa cell line.
348 Comparing to ellipticine, the extracts revealed medium activity. Nevertheless, ellipticine has a
349 very strong inhibiting power on all presented tumor cell lines, but also exhibits high
350 hepatotoxicity to non-tumor PLP2 cell line. In our case, only root showed mild hepatotoxicity
351 towards PLP2 (GI_{50} = 252 ± 10 μg/mL), however it did not exceed active concentrations against
352 the tumor cell lines (40 ± 3 μg/mL in HepG2; 52 ± 5 μg/mL in MCF-7; 113 ± 5 μg/mL in NCI-
353 H460; and 206 ± 15 μg/mL in HeLa).
354 Consequently, although the leaf and root extracts of *S. stramoniifolium* could be useful in the
355 development of new anticancer products, the leaf is the most promising part, since it did not
356 present unspecific toxicity, as suggested by results obtained with PLP2 assay.
357 Due to the possible synergic effect of present compounds, the plant crude extracts can often be a
358 more powerful antioxidant tool than individual substances. Moreover, the natural matrices in
359 form of crude extracts possess usually very low toxicity comparing to individual chemicals and
360 therefore are currently experiencing a renaissance in both phytopharmacological and food
361 industry.[31]
362
363 **4. Conclusions**

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

377

378 **Conflict of interest:**

379 No conflict of interest.

380

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389

390 **References**

391 1 M. Carocho and I. C. F. R. Ferreira, *Food Chem. Toxicol.*, 2013, **51**, 15–25.

392 2 O. Paredes-Lopez, M. L. Cervantes-Ceja, M. Vigna-Perez and T. Hernandez-Perez, *Plant*
393 *Foods Hum. Nutr.*, 2010, **65**, 299–308.

394 3 D. A. Herms and W. J. Mattson, *Q. Rev. Biol.*, 1992, **67**, 283–335.

395 4 M. D. Whalen, D. E. Costich and C. B. Heiser, *Gentes Herbarum*, 1981, **12**, 41–129.

396 5 H. B. Das, K. Majumdar, B. K. Datta and D. Ray, *Nat. Prod. Radiance*, 2009, **8**, 172–180.

397 6 R. A. DeFilipps, S. L. Maina and J. Crepin, *Medicinal Plants of the Guianas (Guyana,*
398 *Surinam, French Guiana)*, Department of Botany, National Museum of Natural History,
399 Smithsonian Institution Washington, D. C., 2004, 491 p.

400 7 C. T. Pedrollo, V. F. Kinupp, G. Shepard Jr and M. Heinrich, *J. Ethnopharmacol.*, 2016,
401 **186**, 111–124.

402 8 Y. Estevez, D. Castillo, M. T. Pisango, J. Arevalo, R. Rojas, J. Alban, E. Deharo, G.
403 Bourdy and M. Sauvain, *J. Ethnopharmacol.*, 2007, **114**, 254–259.

404 9 W. Samee, M. Engkalohakul, N. Nebbua, P. Direkrojanavuti, C. Sornchaithawatwong and
405 N. Kamkaen, 2006, **1**, 196–203.

406 10 P. Wetwitayaklung and T. Phaechamud, *Res. J. Pharm. Biol. Chem. Sci.*, 2011, **2**, 146–
407 154.

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View Article Online
DOI: 10.1039/C7FO00297A

Published on 21 April 2017. Downloaded by Middle East Technical University (Orta Dogu Teknik U) on 21/04/2017 10:58:13.

408 11 A. Sakunpak and P. Panichayupakaranant, *Food Chem.*, 2012, **130**, 826–831.

409 12 R. Sarnthima and S. Khammuang, *Int. J. Agric. Biol.*, 2012, **14**, 111–115.

410 13 I. C. S. Aires, R. A. Lima and A. G. S. Braga, in *64º Congresso Nacional de Botânica*,
411 Belo Horizonte, Brazil, 2013.

412 14 R. Pingal, Thesis, University of West Indies, 2008.

413 15 PAHO/WHO, 2013.
414 http://www.paho.org/hq/index.php?option=com_content&view=article&id=9135%3A201
415 3-cancer-mortality-declining-some-countries-americas-new-paho-who-
416 report&catid=740%3Apress-releases&Itemid=1926⟨=en, (accessed May 2016)

417 16 H. Chandoura, J. C. M. Barreira, L. Barros, C. Santos-Buelga, I. C. F. R. Ferreira and L.
418 Achour, *Ind. Crops Prod.*, 2015, **65**, 383–389.

419 17 L. Barros, E. Pereire, R. C. Calhelha, M. Duenas, A. M. Carvalho, C. Santos-Buelga and I.
420 C. F. R. Ferreira, *J. Funct. Foods*, 2013, **5**, 1732–1740.

421 18 CLSI, *Performance Standards for Antimicrobial Susceptibility Testing, 18th informational*
422 *supplement. CLSI document M100-S18*, Clinical and Laboratory Standards Institute,
423 Wayne, PA, USA, 2008.

424 19 EUCAST, *European Society of Clinical Microbiology and Infectious Diseases*, 2013.
425 http://www.eucast.org/ast_of_bacteria/previous_versions_of_documents/, (accessed March
426 2016).

427 20 V. Kuete, P. Y. Ango, G. W. Fotso, G. D. W. F. Kapche, J. P. Dzoyem, A. G. Wouking, B.
428 T. Ngadjui and B. M. Abegaz, *BMC Complement. Altern. Med.*, 2011, **11**.

Food & Function Accepted Manuscript

View Article Online
DOI: 10.1039/C7FO00297A

Published on 21 April 2017. Downloaded by Middle East Technical University (Orta Dogu Teknik U) on 21/04/2017 10:58:13.

429 21 J. Pinela, L. Barros, M. Dueñas, A. M. Carvalho, C. Santos-Buelga and I. C. F. R. Ferreira,
430 *Food Chem.*, 2012, **135**, 1028–1035.
431 22 R. C. G. Correa, A. Henrique Pereira de Souza, R. C. Calhelha, L. Barros, J. Glamoclija,
432 M. Sokovic, R. M. Peralta, A. Bracht and I. C. F. R. Ferreira, *Food Funct.*, 2015, **6**, 2155–
433 2164.
434 23 R. M. V Abreu, I. C. F. R. Ferreira, R. C. Calhelha, R. T. Lima, M. Helena Vasconcelos,
435 F. Adega, R. Chaves and M.-J. R. P. Queiroz, *Eur. J. Med. Chem.*, 2011, **46**, 5800–5806.
436 24 L. Barros, M. Dueñas, A. M. Carvalho, I. C. F. R. Ferreira and C. Santos-Buelga, *Food*
437 *Chem. Toxicol.*, 2012, **50**, 1576–1582.
438 25 M. N. Clifford, K. L. Johnston, S. Knight and N. Kuhnert, *J. Agric. Food Chem.*, 2003, **51**,
439 2900–2911.
440 26 M. N. Clifford, J. Kirkpatrick, N. Kuhnert, H. Roozendaal and P. R. Salgado, *Food Chem.*,
441 2008, **106**, 379–385.
442 27 A. J. Parr, F. A. Mellon, I. J. Colquhoun and H. V Davies, *J. Agric. Food Chem.*, 2005, **53**,
443 5461–5466.
444 28 A. L. Gancel, P. Alter, C. Dhuique Mayer, J. Ruales and F. Vaillant, *J. Agric. Food Chem.*,
445 2008, **56**, 11890–11899.
446 29 C. E. Narváez-Cuenca, J. P. Vincken and H. Gruppen, *Food Chem.*, 2012, **130**, 730–738.
447 30 G. Normanno, G. La Salandra, A. Dambrosio, N. C. Quaglia, M. Corrente, A. Parisi, G.
448 Santagada, A. Firinu, E. Crisetti and G. V Celano, *Int. J. Food Microbiol.*, 2007, **115**, 290–
449 296.

Food & Function Accepted Manuscript

View Article Online
DOI: 10.1039/C7FO00297A

Published on 21 April 2017. Downloaded by Middle East Technical University (Orta Dogu Teknik U) on 21/04/2017 10:58:13.

450 31 M. Carocho, M. F. Barreiro, P. Morales and I. C. F. R. Ferreira, *Compr. Rev. Food Sci.*
451 *Food Saf.*, 2014, **13**, 377–399.

452 32 J. N. Pendleton, S. P. Gorman and B. F. Gilmore, *Expert Rev. Anti. Infect. Ther.*, 2013, **11**,
453 297–308.

454 33 D. I. Andersson and D. Hughes, *Nat. Rev. Microbiol.*, 2010, **8**, 260–271.

455 34 A. Kröner, N. Marnet, D. Andrivon and F. Val, *Plant Physiol. Biochem.*, 2012, **57**, 23–31.

456 35 A. Winczura, D. Zdzalik and B. Tudek, *Free Radic. Res.*, 2012, **46**, 442–459.

457 36 A. M. Pisoschi and A. Pop, *Eur. J. Med. Chem.*, 2015, **97**, 55–74.

458 37 R. L. Prior, X. L. Wu and K. Schaich, *J. Agric. Food Chem.*, 2005, **53**, 4290–4302.

459 38 B. N. Ames, M. K. Shigenaga and T. M. Hagen, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**,
460 7915–7922.

461 39 E. Elisabetsky and G. C. de Souza, *Farmacogn. da planta ao Medicam.*, 2004, **2**, 87–99.

462 40 M. Amir and S. Kumar, *J. Sci. Ind. Res.*, 2004, **63**, 116–124.

463 41 K. W. Kuo, S. H. Hsu, Y. P. Li, W. L. Lin, L. F. Liu, L. C. Chang, C. C. Lin, C. N. Lin
464 and H. M. Sheu, *Biochem. Pharmacol.*, 2000, **60**, 1865–1873.

465

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Table 1. Ethnomedicinal information on *Solanum stramoniifolium* Jacq.

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

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Table 2. Resistance profile of Gram-negative bacteria to different antibiotics; MIC values (µg/ml).

Antibiotics	***A. baumannii***		***E. coli***		***E. coli* ESBL**		***K. pneumoniae***		***K. pneumoniae* ESBL**		***P. aeruginosa***	
Ampicilin	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
Cefuroxim	na		≤4	S	na		>8	R	≥64	R	na	
Cefotaxim	>32	R	≤1	S	na		>2	R	≥64	R	na	
Ceftazidim	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/Sulfamethoxazol	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

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Table 3. Resistance profile of Gram-positive bacteria to different antibiotics; MIC values (µg/ml).

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	
Ceftarolin	≤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/Sulfamethoxazol	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.

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Table 4A. 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 identificaion	Quantification (mg/g 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

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Table 4B. 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 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

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Table 5. Antibacterial activity of *Solanum stramoniifolium* hydroethanolic extracts (MIC; mg/mL).

Bacteria	MIC (mg/mL)		
	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*

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Table 6. Biological activity of hydroethanolic extracts from different parts of *Solanum stramoniifolium* Jacq.

Antioxidant activity (EC_{50} values, µg/mL)				
	Leaf	**Root**	**Stem**	**Trolox**
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
Anti-inflammatory activity (EC_{50} values, µg/mL)				
	Leaf	**Root**	**Stem**	**Dexamethasone**
Nitric oxide (NO) production	>400	100±6	>400	16±1
Cytotoxicity to tumor cell lines (GI_{50} values, µg/mL)				
	Leaf	**Root**	**Stem**	**Ellipticine**
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)				
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 ($p < 0.05$).

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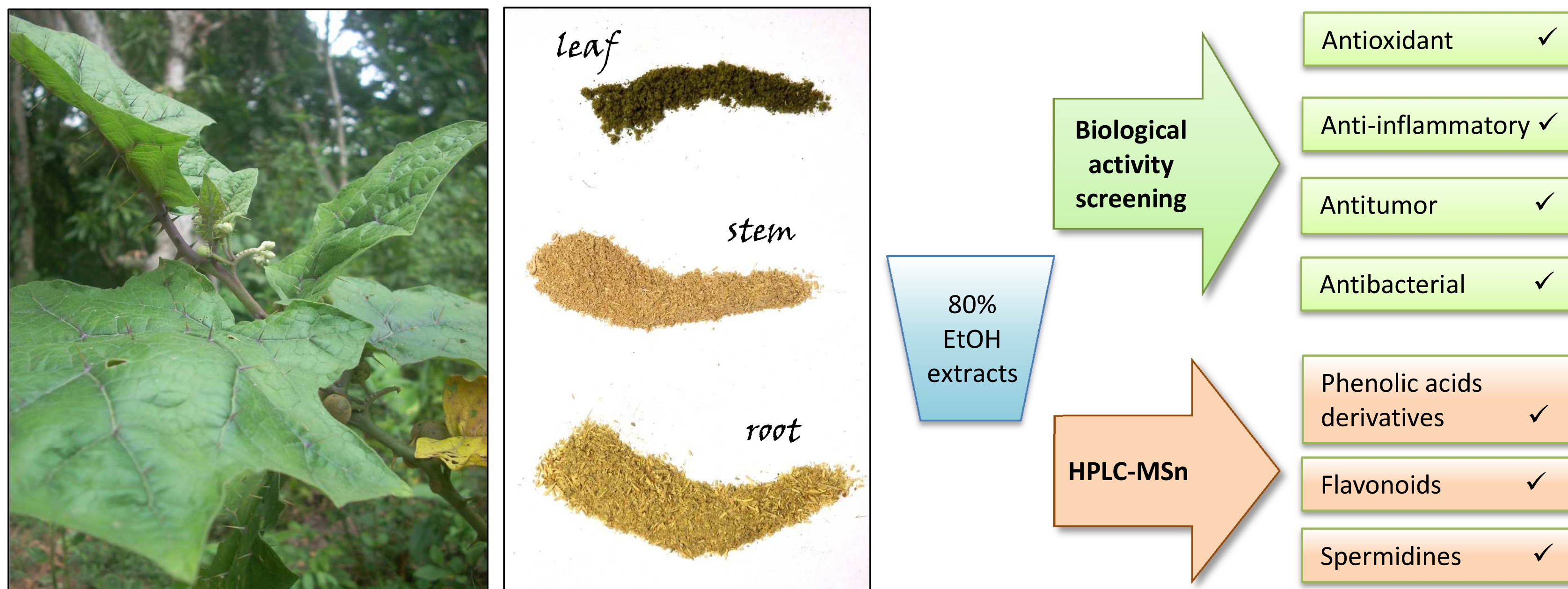
Graphical Abstract

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

Blanka Svobodova, Lillian Barros, Tomas Sopik, Ricardo C. Calhelha, Sandrina Heleno, Maria Jose Alves, Simone Walcott, Vlastimil Kuban, Isabel C.F.R. Ferreira



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