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Male sexual enhancers from the Peruvian Amazon

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ABSTRACT

Ethnopharmacological relevance: Selected Peruvian Amazon plants are macerated into sugar cane distillates to prepare alcoholic beverages used to improve male sexual performance. The tree bark from *Campsiandra angustifolia* Spruce ex Benth (Fabaceae), *Swartzia polyphylla* DC (Fabaceae), *Minquartia guianensis* Aubl. (Olacaceae) and *Thynantus panurensis* (Bureau) Sandwith (Bignoniaceae) usually are used as crude drugs in mixtures of several ingredients.

Aim of study: Describe the chemical composition of the most traded traditional male enhancer beverages, namely "Levántate Lazaro" and "Siete veces sin sacarla", and their single crude drug constituents, as well as their inhibitory activity towards the enzyme phosphodiesterase-5. The presence of pro-sexual drugs such as Sildenafil® and derivatives was assessed in the samples.

Materials and methods: Single plant constituents and the preparation mixtures were purchased in the Mercado Belen (Iquitos, Peru). Chemical profiling was carried out by HPLC-DAD-ESI-MS/MS. The extracts were assessed for phosphodiesterase-5 inhibition. The occurrence of pro-sexual drugs was determined by HPLC-DAD-ESI-MS/MS.

Results: Chemical profiling allowed the identification of condensed tannins as the main constituents of *C. angustifolia* and *S. polyphylla*, hydrolysable tannins for *M. guianensis*, and *C*-glycosides for *T. panurensis*. The traditional preparations showed similar composition compared to the crude drugs. At 200 μg/mL, the traditional preparation "Levántate Lázaro" and "Siete veces sin sacarla" inhibited the phosphodiesterase-5 by 49.88% and 27.90%, respectively. No adulterations with pro-sexual drugs were found in the samples. From the crude drugs, low effect was found for the extracts of *S. polyphylla* and *T. panurensis* and high activity for *C. angustifolia* which inhibited the enzyme by 89.37% and 81.32% at 200 and 100 μg/mL, respectively.

Conclusion: The traditional preparations used to improve sexual performance in the Peruvian Amazon showed activity as phosphodiesterase-5 inhibitors. The most active ingredient of the traditional preparations was *C. angustifolia*, with some contribution from *T. panurensis*. These results encourage additional studies, including animal models to confirm the male enhancer effect of the preparations.

1. Introduction

The use of crude drugs to improve sexual performance is a known practice worldwide. These include plant and animal parts, and are often associated with cultural beliefs (Sharma et al., 2017; van Andel et al., 2012; Melnyk and Marcone, 2011; Drewes et al., 2003). In a review on sexual enhancers, West and Krychman (2015) revised the products marketed in the USA as aphrodisiacs. They included products from plant and animal origin, as well as vitamins and minerals. However, little information is available on the proven efficacy of the products on

Abbreviations: Ca:, *Campsiandra angustifolia*; HHDP, hexahydroxydiphenic acid; HPLC-DAD-MS, high performance liquid chromatography coupled to diode array detector and mass spectrometry; LL, Levántate Lázaro; *Mg:*, *Minquartia guianensis*; PDE-5, phosphodiesterase-5; *Sp:*, *Swartzia polyphylla*; SVSS, siete veces sin sacarla; TIC, Total ion chromatogram; *Tp:*, *Tynanthus panurensis*

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randomized clinical trials. From the products described, *Lepidium meyenii* (maca) is native of the high mountains of Peru. Beharry and Heinrich (2018) revised the literature on the reproductive benefits of this Andean root. The results, so far, have provided inconclusive results and more research in needed using different approaches. However, a study using a commercial extract showed some effect on latent penile erection time in rats (Zheng et al., 2000).

In the Peruvian Amazon, with a very rich knowledge derived from Native Americans, the use of animal and plant drugs for different purposes is widespread. The traditional use of sexual enhancers in ethanol-containing beverages as solvent, comes from the times of rubber tree (*Hevea brasiliensis*) exploitation in the second half of the 19th century. The use of male aphrodisiac preparations by the Peruvian Amazon communities is common and well-established in the local lore, as well as in the literature written by the Nobel laurate Mario Vargas-Llosa (Fariña-Pérez and Meijide-Rico, 2015). Animal drugs are also used as sexual enhancers in the Peruvian Amazon. The terrapin penis is used in childbirth, and the oil from the freshwater dolphin is reputed as a sexual attractant, among others (Schmeda-Hirschmann et al., 2014).

The two most consumed aphrodisiac preparations in Iquitos as reported in the traditional market, are SVSS and LL (Fig. 1). The acronyms SVSS stands for “siete veces sin sacarla” (seven times without taken it out) and LL for “Levántate Lázaro” (rise up Lazarus!), referring to the effect that they are supposed to elicit in the male consumers. LL and SVSS are prepared using mixtures of Amazonian plants (tree bark) as crude drugs and “aguardiente”, which is a sugar cane distillate as the solvent. The coarsely ground plant parts, in definite ratios, are macerated in “aguardiente”, containing 18–20% ethanol. Beehoney is added to improve the taste of the traditional preparation.

The crude drugs used to prepare the above mentioned beverages are the bark from the trees: *Campsiandra angustifolia* Spruce ex Benth (Leguminosae) “huacapurana”, *Minquartia guianensis* Aubl. (Olacaceae) “fierro caspi”, *Swartzia polyphylla* DC (Fabaceae) “cumaceba” and *Tynanthus panurensis* (Bureau) Sandwith (Bignoniaceae) “clavo huasca”. LL and SVSS contain three different bark sources. For SVSS, the bark of *T. panurensis*, *S. polyphylla* and *C. angustifolia* are used. According to the sellers, the SVSS tonic is recommended to “improve energy” and as a reconstituent. It is made by placing together a handful of each dry and sliced bark in about 1 L of “aguardiente”. The barks are macerated for about 20 days. Then, the preparation is filtered and bottled. LL differs from SVSS by replacing *C. angustifolia* with *M. guianensis*. LL is used as male sexual enhancer and to relieve bone pain, usually associated with cold. It is also recommended for tiredness and apathy. The daily dose for both preparations is to drink a small glass of about 20–50 mL with an empty stomach. If the body “become too hot”, a shower is recommended.

The discovery and commercialization of sildenafil citrate (Viagra®) and related products was a landmark in the efficient treatment of penile erection deficiency. The pharmacodynamics of sildenafil is the inhibition of phosphodiesterase-5, and may represent one of the possible mechanisms of action explaining the effect of the traditional preparations used as male aphrodisiacs. Phosphodiesterases (PDE) are important enzymes participating in the regulation of all cellular functions. They hydrolyze the phosphodiester bond of cyclic adenoside monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP), increasing the intracellular concentrations of these second messengers. A PDE inhibitor can increase the intracellular concentration of cAMP or cGMP depending on the particular substrate specificity (Bischoff, 2004). Eleven PDEs have been described due to their substrate specificity, kinetic properties, tissue and subcellular localization, drug and mediator sensitivities (Abusnina and Lugnier, 2017). PDE-5 is a predominant cGMP metabolizing enzyme and is expressed in the cavernosal tissue and in penile arteries. This enzyme is the pharmacological target of sildenafil (Viagra®), vardenafil (Levitra ®) and tadalafil (Cialis ®) in the treatment of erectile dysfunction (Corbin and Francis, 2003).

The aim of the present work was to describe the chemical composition of the traditional preparations and their crude drug constituents, commonly used as male sexual enhancers in the Peruvian Amazon (Iquitos, Peru). In addition, the inhibitory effect of these crude drugs and preparations were assessed towards PDE-5.

2. Materials and methods

2.1. Traditional preparation and crude drugs

Interviews with the traditional sellers at the Iquitos market allowed the identification of the native trees used as crude drugs to prepare the male sexual enhancers. The plants were identified by Mr. Juan Ruiz Macedo, botanist from the Herbarium Amazonense (UNAP) where the voucher herbarium specimens are kept. The plants were identified as follows: *Campsiandra angustifolia* Spruce ex Benth (Fabaceae) (0001411), *Minquartia guianensis* Aubl. (Olacaceae) (0028687), *Swartzia polyphylla* DC (Fabaceae) (39437) and *Tynanthus panurensis* (Bureau) Sandwith (Bignoniaceae) (39133). In all the cases, the tree bark is used for the preparations.

The traditional preparations sold under the commercial names “Levántate Lázaro” (LL) and “Siete veces sin sacarla” (SVSS) were purchased at the Pasaje Paquito, Mercado Belén, Iquitos, Perú, in September 2014. The preparations were filtered and concentrated under reduced pressure to remove the ethanol (about 18–20% EtOH). Then, the concentrated samples were dissolved in distilled water (2 L)

Fig. 1. a) Traditional preparations sold as sexual enhancers in the Iquitos Markets, Peruvian Amazon. The crude drug mixtures are macerated into a sugar cane distillate with 15–20% ethanol. The different names in the labels refer to the supposed sexual enhancing effect of the preparations. b) The preparations investigated: “siete veces sin sacarla” (SVSS) and “levántate lázaro” (LL).

and the phenolic and other polar constituents were adsorbed using activated Amberlite XAD-7 resin (Sigma-Aldrich, St. Louis, MO, USA). After stirring the resin with the aqueous solution for 1 h, the resin was separated by filtering, washed with distilled water (3 × 1 L). The adsorbed compounds were desorbed using MeOH (3 × 0.5 L) and acetone (2 × 0.25 L). The combined enriched extracts of each preparation were separately taken to dryness to calculate the extraction yields, based on the volume of the preparation. Samples of the different crude drugs used for the recipes (*C. angustifolia*, *M. guianensis*, *S. polyphylla* and *T. panurensis*) were powdered and extracted with MeOH and MeOH:H_2O (1:1) to afford the crude extracts for comparison and fingerprint analysis.

2.2. HPLC-DAD-ESI-MSn analysis

The extracts were compared by HPLC-DAD to establish similarities and differences among the plant species, to characterize the fingerprints of the preparations and to set-up the conditions for HPLC-MS/MS analysis. The Amberlite XAD-7-retained extracts from the commercial preparations and single constituents of LL and SVSS were analyzed with a Shimadzu Prominence equipment (Shimadzu Co., Kyoto, Japan). The HPLC was equipped with an LC-20AT pump, SPD-M20A UV diode array detector and CTO-20AC column oven, and a Labsolution software. A MultoHigh 100 RP 18–5 μm (250 × 4.6 mm) column (CS-Chromatographie Service Gmbh., Langerwehe, Germany) was used. Approximately 10 mg of each extract were dissolved in 1 mL MeOH, filtered through a 0.45 μm PTFE filter (Waters, Milford, MA, USA) and injected. The solvent system used consisted of H_2O-formic acid (99:1, v/v, solvent A) and acetonitrile (solvent B) as follows: 10–22% B from 0 to 40 min, 22–32% B from 40 to 55 min, 32–38% B from 55 to 70 min, 38–55% B from 70 to 80 min, 55–80% B from 80 to 85 min and returning to 10% B from 85 to 90 min. The initial conditions were maintained for extra 5 min to stabilize the column between injections. Compounds were monitored at 254, 280 and 330 nm. Spectra from 200 to 600 nm were recorded for peak characterization. The flow rate was 1 mL/min, the column oven was set at 25 °C, and the injected volume was 20 μL.

Mass spectra were recorded by means of an Agilent 1100 (Agilent Technologies Inc., CA, USA) liquid chromatographer in line with an Esquire 4000 Ion Trap LC/MS(n) system (Bruker Daltonics, Billerica, MA, USA) through a split connection. Ionization was performed at 3000 V employing nitrogen as nebulizing and drying gas at 50 psi and 365 °C, with a flow rate of 10 L/min. Negative ions were detected through full scan mode (*m/z* 20-2200) with normal resolution (scan speed 10,300 *m/z*/s; peak with 0.6 FWHM/*m/z*). The trap parameters were set in ion charge control (ICC) using the manufacturer default parameters, and a maximum accumulation time of 200 ms. The mass spectrometric conditions for negative ion mode were: electrospray needle, 4000 V; end plate offset, −500 V; skimmer 1, 56.0 V; skimmer 2, 6.0 V; capillary exit offset, 84.6 V; capillary exit, 140.6 V. The fragmentation amplitude for the collision-induced dissociation (CID) spectra was set at 1.00 V (MS/MS) employing helium as collisional gas and was automatically controlled through SmartFrag option.

The presence of pro-sexual drugs in the samples was assessed using sildenafil citrate standard for Rt matching in the same chromatographic method used for the samples. The *m/z* value of the commercial drugs sildenafil (*m/z* 473), vardenafil (*m/z* 487), tadalafil (*m/z* 389), and their derivatives homosildenafil (*m/z* 487), hydroxyhomosildenafil (*m/z* 503), piperidino sildenafil (*m/z* 458), aildenafil (*m/z* 487), thiosildenafil (*m/z* 489), thiohomosildenafil (*m/z* 503), thioaildenafil (*m/z* 503), acetildenafil (*m/z* 465), noracetildenafil (*m/z* 451), hydroxyacetildenafil (*m/z* 481), piperidinoacetildenafil (*m/z* 436), piperidenafil (*m/z* 458), aminotadalafil (*m/z* 389) and benzamidenafil (*m/z* 388) were searched in the EIC, following the directions of Singh et al. (2009).

2.3. Inhibition of phosphodiesterase-5 (PDE-5)

The inhibition of PDE-5 was carried out following the instructions provided in the commercial kit (ab139460, Abcam, Cambdrige, UK). The principle of the assay is the cleavage of 3′,5′-cGMP into the free nucleotide, which is then cleaved into the nucleoside and phosphate by a 5′-nucleotidase. The phosphate released is then quantified using the Green Assay Reagent provided by the supplier. Samples (200 and 100 μg/mL) or sildenafil citrate (0–50 μg/mL) were incubated with 3′-5′-cGMP and 5′-nucleotidase in the appropriate assay buffer. Then, PDE-5 was added to the wells and incubated at 37 °C for 30 min. At the end of the incubation, 100 μL of the Green Assay Reagent were added and further incubated for 30 min at room temperature. At the end of the incubation, absorbance was read at 600 nm in a microplate reader (ELX800, Biotek, Winooski, VT, USA). The results are expressed as the concentration of sample that inhibits PDE-5 by 50% (IC_{50}, μg/mL) or percent of inhibition at 100 and 200 μg/mL.

3. Results

3.1. Chemical profiles of the crude drug and preparations

The traditional Peruvian preparations LL and SVSS as well as the ingredients *C. angustifolia*, *M. guianensis*, *S. polyphylla* and *T. panurensis* were separately investigated to establish the composition of the single crude drugs and the commercial mixtures. The w/v yields from the hydroalcoholic preparations were 0.48% and 0.53%, for the beverages LL and SVSS, respectively. The w/w extraction yields of the crude drug constituents were 10.5%, 11.6, 18.9% and 14.3% for *C. angustifolia*, *M. guianensis*, *S. polyphylla* and *T. panurensis*, respectively.

The extracts from the traditional preparations and crude drugs were analyzed by HPLC-DAD-ESI-MS^n. The HPLC profiles of the crude drugs are depicted in Fig. 2. The fingerprints from SVSS and LL are shown in Fig. 3. The main signals of the preparations overlap with some of the major compounds from the crude drug profiles. This suggests that the crude drugs were indeed employed as ingredients for the aphrodisiac preparations, as mentioned by the sellers. The tentative identification of the compounds is described in the following paragraphs.

3.1.1. Campsiandra angustifolia (“huacapurana”)

The chemical profile of *C. angustifolia* is depicted in Fig. 2a. The HPLC-MS analysis allowed the tentative identification of 31 compounds, including a caffeoylquinic acid, 25 proanthocyanidins and gallotannins, four flavonoids and one unidentified compound (Table 1).

Compound **1** presented a base peak ion at *m/z* 353 amu. The neutral loss of a caffeoyl moiety lead to a MS^2 base peak at *m/z* 191 amu, and a MS^3 fragment at 179 amu. The hierarchical scheme proposed by Clifford et al. (2003) allowed the tentative identification of compound **1** as 5-caffeoylquinic acid. Compounds **2**, **4**, **5**, **6**, **7**, **8**, **10**, **12**, **15** and **18** showed a common base peak ion at *m/z* 561 amu, with characteristic MS^2 fragments at *m/z* 543, 451, 409 and a main peak at *m/z* 289 amu. This fragmentation pattern is characteristic of proanthocyanidin dimers based on (*epi*)-afzelechin and (*epi*)-catechin (Lin et al., 2014). Compounds **23** and **26** showed a common base peak ion at *m/z* 713 amu, with a main MS^2 fragment at 561 [M-H-galloyl]. The similar pattern of fragmentation allowed the identification of these compounds as galloyl-(*epi*)-afzelechin-(*epi*)-catechin isomers (Sobeh et al., 2018). Compound **11** showed a base peak ion at *m/z* 545, with a neutral loss of 256 amu, leading to a MS^2 base peak at *m/z* 289 amu. The compound was identified as a proanthocyanidin dimer based on (*epi*)-guibourtinidol-(*epi*)-catechin (Sobeh et al., 2018). Compound **14** showed a base peak ion at *m/z* 575 amu, with MS^2 base peak at *m/z* 289 amu. The compound was tentatively identified as A-type procyanidin dimer (Lin et al., 2014).

Compounds **13**, **17**, **20**, **21**, **22** and **25** showed a common base peak at *m/z* 833 amu, with a neutral loss of 272 amu leading to a MS^2 ion at *m/z* 561 amu, indicating the presence of a second (*epi*)-azfelechin

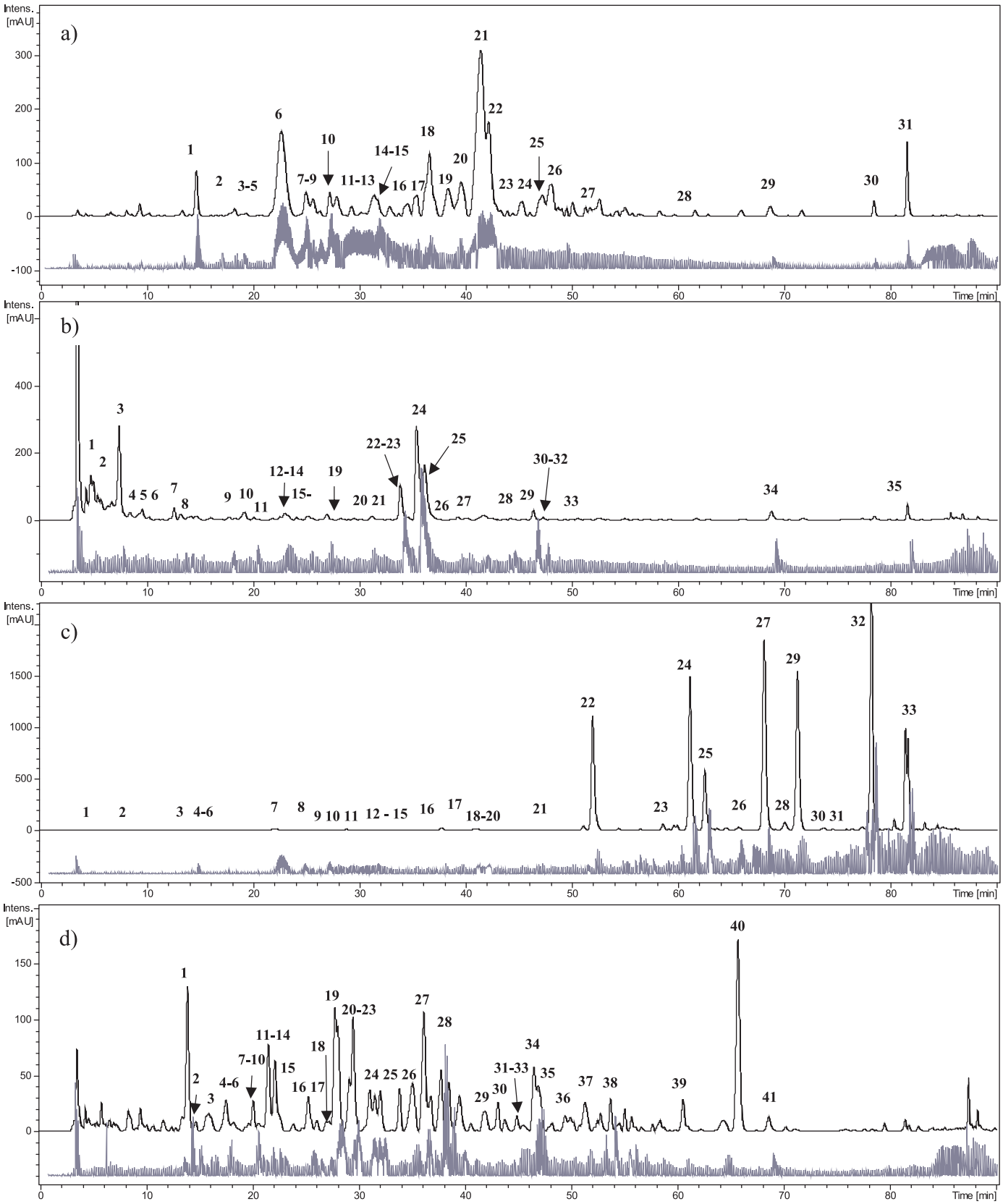

Fig. 2. Total ion chromatogram (TIC, gray) and HPLC profile at 280 nm (black) of the crude drugs used to prepare the sexual enhancing beverages. a) *Campsiandra angustifolia*; b) *Minquartia guianensis*; c) *Swartzia polyphylla*; d) *Tynanthus panurensis*. The compounds identification in the single crude drugs is presented in Tables 1–4, respectively.

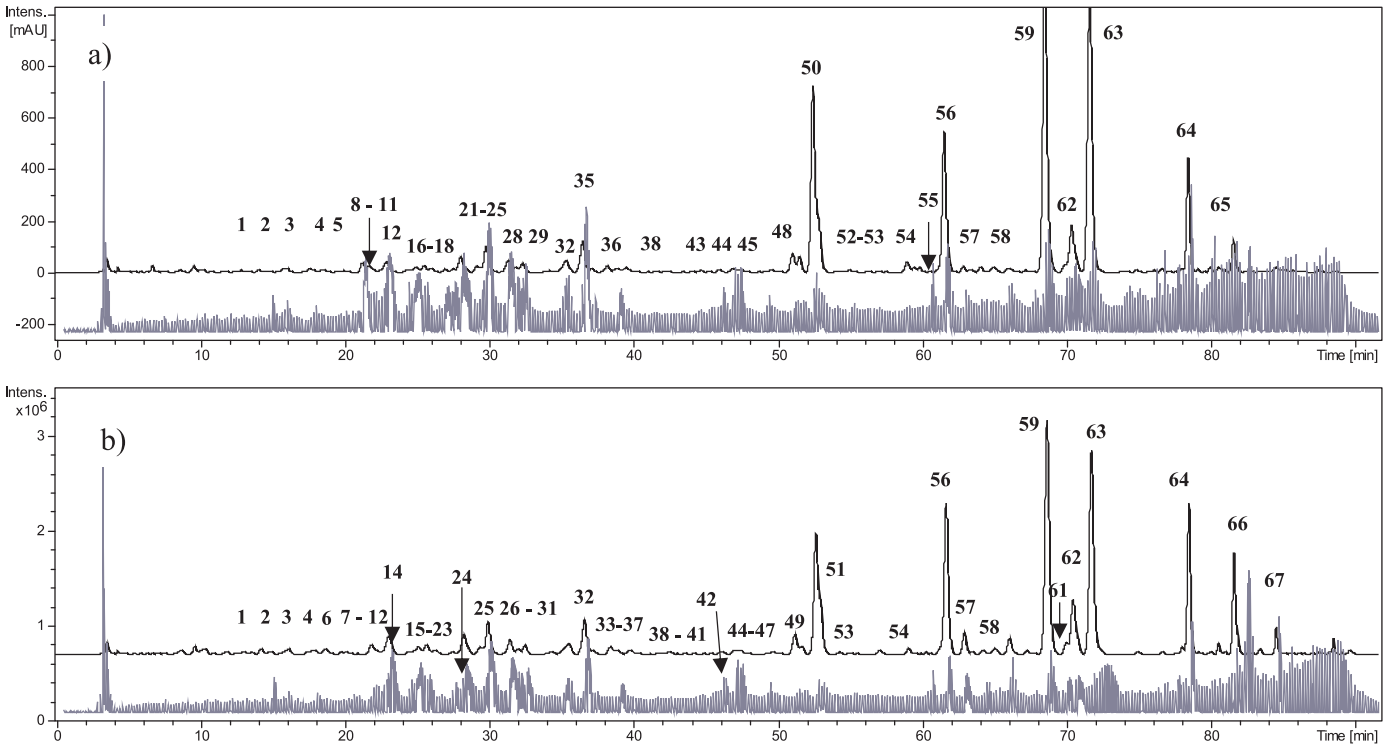

Fig. 3. Total ion chromatogram (TIC, gray) and HPLC profile at 280 nm (black) of the aphrodisiac preparations: a) "levántate lázaro" (LL); b) "siete veces sin sacarla" (SVSS). The compound identity in the preparations is summarized in Table 5.

Table 1
Tentative identification of compounds in the extract from *Campsiandra angustifolia* by HPLC-ESI-MS/MS in the negative ion mode.

Peak	Rt (min)	$[M-H]^-$	λ max	MS^2 fragments (% base peak intensity)	Tentative identification
1	13.8	353.5	323	190.5 (100), 178.40 (13)	5-caffeoylquinic acid (Clifford et al., 2003)
2	16.7	561.55		543.02 (18), 450.96 (74), 409.06 (81), 288.97 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 1
3	17.3	633.8		481.6 (39), 300.8(100), 274.70 (20)	Galloyl-HHDP-hexoside
4	18.1	562.1		408.9 (62), 288.90 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 2
5	18.8	561.5		543.0 (18), 451.0 (69), 409.4 (98), 289.1 (98)	(*epi*)-afzelechin-(*epi*)-catechin dimer 3
6	22.2	561.1	280, 239	409.1 (91), 288.9 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 4
7	24.7	561.2	281,239	408.9 (83), 288.9 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 5
8	25.2	561.3	281,239	409.1 (97), 288.9 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 6
9	25.4	833.3		681.1 (61), 529.7 (100), 289.2 (6)	B-type proanthocyanidin trimer
10	26.9	561.3		409.1 (100), 288.9 (79)	(*epi*)-afzelechin-(*epi*)-catechin dimer 7
11	27.3	545.7		527.1 (19), 435.1 (39), 409.1 (34) 288.9 (100)	(*epi*)-guibourtinidol-(*epi*)-catechin dimer (Sobeh et al., 2018)
12	29.0	561.2		409.1 (81), 288.9 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 8
13	30.9	833.1		681.2 (100), 561.7 (85), 530.8 (71), 289.0 (8)	(*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 1
14	33.2	575.2		422.8 (100), 406.9 (34), 288.7 (15)	A-type procyanidin dimer
15	33.8	561.3		409.0 (100), 288.9 (83)	(*epi*)-afzelechin-(*epi*)-catechin dimer 9
16	35.2	609.6	360,	300.8 (100)	Quercetin rutinoside
17	35.5	833.3		681.3 (60), 529.9 (100), 289.3 (8)	(*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 2
18	36.2	561.4	281, 240	451.0 (82), 408.9 (86), 288.8 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 10
19	38.3	817.8		664.9 (16), 560.9 (100), 409.0 (20), 288.8 (9)	Cassanidin A isomer 1 (Erasto and Majinda, 2003)
20	39.1	833.3	281, 241	681.2 (100),561.2 (67), 529.9 (55), 289.4 (5)	(*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 3
21	41.1	833.5	281, 241	681.1 (57), 561.4 (60), 529.9 (100)	(*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 4
22	41.9	833.4		681.1 (100), 561.9 (57), 530.3 (62), 289.3 (4)	(*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 5
23	43.6	712.7		561.1 (100), 543.2 (53), 391.2 (12), 271.0 (14)	Galloyl-(*epi*)-afzelechin-(*epi*)-catechin isomer 1
24	43.7	817.6		561.3 (100), 409.3 (8), 289.3 (3)	Cassanidin A isomer 2 (Erasto and Majinda, 2003)
25	47.3	833.6		681.2 (100), 561.6 (64), 530.9 (73), 289.3 (5)	(*epi*)-afzelechin-(epi)-afzelechin-(*epi*)-catechin trimer 6
26	49.1	712.9		561.0 (100), 543.0 (43), 271.0 (14)	Galloyl-(*epi*)-afzelechin-(*epi*)-catechin isomer 2
27	52.0	801.2		765 (6), 691.0 (54), 561.1 (100), 545,2 (90), 409.2 (28), 288.9 (25)	(*epi*)-guibourtinidol-(*epi*)-guibourtinidol-(*epi*)-catechin (Sobeh et al., 2018)
28	61.1	271.2		176.3 (27), 160.4 (8) 150.3 (100)	Naringenin (Molina-Calle et al., 2015)
29	68.3	495.4		451.1 (100), 407.9 (17)	Unknown
30	78.1	285.2		269.7(100)	Hydroxygenistein
31	81.2	282.9	330, 265	267.7(100)	Wogonin

substituent at the proanthocyanidin dimer core. The compounds were identified as proanthocyanidin trimer isomers based on (*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin (Lin et al., 2014). Compound **9** also showed a base peak ion at *m/z* 833 amu, but with a fragmentation pattern leading to a MS^2 ion at 529 amu. The compound was tentatively identified as a B-type proanthocyanidin trimer (Lin et al., 2014). Compounds **19** and **24** showed a common base peak ion at *m/z* 817 amu, with MS^2 fragments at *m/z* 665, 409 and 289 amu, in agreement with cassanidin A (Sobeh et al., 2018). Both compounds were tentatively identified as cassanidin A isomers. Compound **27** showed a base peak ion at *m/z* 801 amu, with characteristic MS^2 fragments at *m/z* 765, 545, 409 and 289, in agreement with (*epi*)-guibourtinidol-(*epi*)-guibourtinidol-(*epi*)-catechin (Sobeh et al., 2018).

Compound **3** showed a base peak ion at *m/z* 633 amu, yielding product ions at 481 [M-H-152], in agreement with the loss of a galloyl group, and the MS^2 base peak ion at 301 amu, corresponding to the lactonization of ellagic acid into an hexahydroxydiphenoyl (HHDP) unit. The compound was tentatively identified as galloyl-HHDP-hexoside (Santos et al., 2011). Compound **16** showed a base peak ion at *m/z* 609, with a MS^2 main peak at *m/z* 301 amu. The compound was tentatively identified as quercetin rutinoside following the mass spectra and UV profile with λ max at 360 nm. Compound **28** showed a base peak ion at *m/z* 271 amu, with MS^2 fragments at 160, 150, 119 and 107 amu, in agreement with naringenin (Molina-Calle et al., 2015). Compound **30** showed a base peak ion at *m/z* 285, with a MS^2 ion at *m/z* 269 amu. The compound was tentatively identified as hydroxygenistein (Chang, 2007). Compound **31** showed a base peak ion at *m/z* 283, with a MS^2 fragment at *m/z* 268. The compound was tentatively identified as the flavone wogonin (Han et al., 2007). Compound **29** showed a base peak ion at *m/z* 495 amu, with a MS^2 fragment at *m/z* 451. The identity of this compound was not determined.

3.1.2. *Minquartia guianensis* (“fierro caspi”)

The chemical profile of *M. guianensis* is shown in Fig. 2b. The tentative identification of the extract constituents is summarized in Table 2. The chromatographic profile shows hydrolysable tannins based on ellagic acid, gallic acid and HHDP, condensed tannins and flavonoids. The neutral losses of ellagitannins during MS^2 fragmentation were assigned as galloyl (152 amu), gallic acid (170 amu), HHDP (302 amu), galloylhexose (332 amu), HHDP hexose (482 amu) and galloyl-HHDP-hexose (634 amu) (Zhu et al., 2015).

The compounds **1** and **5** showed the characteristic fragmentation pathway of an ellagitannin with a $[M-H]^-$ at *m/z* 783 amu and the MS^2 spectrum shows ions at *m/z* 481 and 301. The compounds were assigned as di-HHDP galloyl hexoside (pedunculagin) isomers 1 and 2, respectively (Santos et al., 2011). The related compounds **11** and **14,** with a $[M-H]^-$ of *m/z* 785 amu showed the loss of 152 amu and fragments at 633, 483 and 301, in agreement with HHDP digalloylhexoside (Fracassetti et al., 2013). The compound **4** was characterized by the pseudomolecular ion at *m/z* 933 and the MS^2 ions at *m/z* 631 and 301, being tentatively assigned as galloyl-bis-HHDP hexoside (Castalagin/Vescalagin) (Fracassetti et al., 2013).

The compounds **6**, **10** and **20** were identified as digalloyl-, trigalloyl- and tetragalloyl hexoside, respectively, based on the neutral loss of galloyl $[M-H-152]^-$ and gallic acid units $[M-H-170]^-$, in agreement with Zhu et al. (2015). Five isomers of HHDP-galloyl-hexoside (**2**, **3**, **7**, **8** and **9**), with a base peak ion at *m/z* 633 and MS^2 fragments at *m/z* 463 $[M-H-galloyl]^-$ and 301 amu $[M-H-galloylhexose]^-$ were also identified (Fracassetti et al., 2013).

Several ellagic acid derivatives were identified in *M. guianensis*, including the hexoside (**15**), the glucuronide (**17**), two pentosides (**19** and **22**), the rhamnoside (**24**) and the dihexoside (**25**). All of them showed the characteristic loss of the sugar unit(s), leading to the base

Table 2
Tentative identification of compounds in the extract from *Minquartia guianensis* by HPLC-ESI-MS/MS in the negative ion mode.

Peak	Rt (min)	$[M-H]^-$	λ max	MS^2 fragments (% base peak intensity)	Tentative identification
1	5.3	783.2		481.1 (99), 301.2 (25)	Di-HHDP-hexoside (Pedunculagin) isomer 1 (Fracassetti et al., 2013)
2	5.6	633.4		300.7 (100)	HHDP-galloyl hexoside 1
3	6.9	633.4		300.8 (100)	HHDP-galloyl hexoside 2
4	7.6	933.4		914.9 (5), 631.0 (100), 300.8 (8)	Galloyl-bis-HHDP hexoside (Castalagin/Vescalagin) (Fracassetti et al., 2013)
5	8.1	783.4		480.9 (35), 300.7 (100), 256.8 (7)	Di-HHDP-hexoside (Pedunculagin) isomer 1 (Fracassetti et al., 2013)
6	9.6	483.4		331.0 (14), 312.8(100), 168.4 (14)	Digalloyl hexoside
7	12.9	633.6		300.8 (100)	HHDP-galloyl hexoside 3
8	13.5	633.5		300.8 (100)	HHDP-galloyl hexoside 4
9	17.4	633.3		462.9 (100), 300.9 (100)	HHDP-galloyl hexoside 5
10	19.5	635.2		465.0 (100)	Trigalloyl hexoside
11	20.3	785.2		632.9 (19), 300.7 (100)	HHDP-digalloyl hexoside 1
12	22.3	561.4		409.1 (100), 288.9 (95)	(*epi*)-azfelechin-(*epi*)-catechin dimer 1
13	22.6	335.7		178.4 (100)	Caffeoyl shikimic acid (Ben Said et al., 2017)
14	22.7	785.3		633.0 (22), 483.0 (34), 300.9 (100)	HHDP-digalloyl hexoside 2
15	24.7	463.7		300.6 (100)	Ellagic acid hexoside
16	24.9	561.9		409.1 (68), 288.9 (100)	(*epi*)-azfelechin-(*epi*)-catechin dimer 2
17	25.0	477.9		300.8 (100)	Ellagic acid glucuronide
18	27.1	561.6		409.0 (90), 289.0 (100)	(*epi*)-azfelechin-(*epi*)-catechin dimer 3
19	28.1	433.1		300.5 (100)	Ellagic acid pentoside 1
20	30.8	787.4		617.2 (100), 465.5 (3)	Tetragalloyl hexoside
21	31.7	561.8		409.0 (100), 288.9 (95)	(*epi*)-azfelechin-(*epi*)-catechin dimer 4
22	33.4	433.1	361, 252	300.6 (100)	Ellagic acid pentoside 2
23	34.3	477.3		314.9 (100), 301.0 (3)	Methoxy ellagic acid hexoside
24	35.1	447.0	361, 252	300.2 (100), 300.8 (100), 256.8 (5), 228.6 (4)	Ellagic acid rhamnoside
25	35.9	624.9	366,253	300.8 (100)	Ellagic acid dihexoside
26	36.5	833.5		681.1 (65), 561.3 (95), 529.7 (100), 289.2 (7)	(*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer
27	39.6	452.1		340.9 (100)	Cinchonain Ia
28	43.6	447.6		314.8 (100)	Rhamnetin/Isorhamnetin pentoside
29	46.0	461.1	360, 236	314.8 (100)	Rhamnetin/Isorhamnetin rhamnoside 1
30	46.9	461.3		314.8 (100)	Rhamnetin/Isorhamnetin rhamnoside 2
31	47.1	817.4		665.1 (100), 561.2 (100), 289.1 (13)	(*epi*)-guibourtinidol-(*epi*)-afzelechin-(*epi*)-catechin
32	47.9	463.0		300.8 (100)	Quercetin hexoside
33	50.0	447.5		284.8 (100)	Kaempferol hexoside
34	68.3	495.4		451.1 (100), 407.9 (17)	Unknown
35	81.2	283.1		267.7 (100)	Wogonin

peak at *m/z* 301, corresponding to ellagic acid, as confirmed by the UV absorption spectra with characteristic maxima at 360–366 and 252–253 nm and intensity of the peaks allowing clear differentiation with quercetin. In addition, compound **23**, presented a [M-H]$^-$ ion at 477 amu, with MS^2 base peaks at *m/z* 315 and 301, and was identified as methoxy ellagic acid hexoside.

The compounds **12**, **16**, **18** and **21** were assigned as (*epi*)-azfelechin-(*epi*)-catechin dimers 1–4 based on the [M-H]$^-$ peak at *m/z* 561 and the fragmentation pattern leading to *m/z* 289 amu, characteristic of (*epi*) catechin (Lin et al., 2014). The compound **26**, with [M-H]$^-$ peak at *m/z* 833 clearly shows the presence of an additional (*epi*)-afzelechin moiety and was identified as (*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer. The compound **31**, with [M-H]$^-$ peak at *m/z* 817 shows differences in one of the monomers, being assigned as (*epi*)-guibourtinidol-(*epi*)-afzelechin-(*epi*)-catechin (Sobeh et al., 2018). Cinchonain Ia (**27**) was identified in the extract by the characteristic mass spectra showing the loss of 110 amu from the [M-H]$^-$ peak at *m/z* 451, in agreement with Bernardo et al. (2018).

The compounds **28-30, 32** and **33**, showed the neutral loss of a pentose (**28**), rhamnose (**29** and **30)** and hexoses (**32-33**) leading to the base peak ion at *m/z* 301 amu (compounds **24**, **25, 32**), *m/z* 315 amu (**28–30**) and *m/z* 285 amu (**33**). The compounds were identified as rhamnetin/isorhamnetin pentoside (**28**), rhamnetin/isorhamnetin rhamnoside 1 and 2 (**29** and **30**, respectively), quercetin hexoside (**32**) and kaempferol hexoside (**33**).

Compound **13** was tentatively assigned as caffeoylshikimic acid due to the base peak at *m/z* 335 with MS^2 ions at *m/z* 179, 161 and 135 amu (Ben Said et al., 2017). The flavonoid Wogonin (**35**) was also identified on the basis of the [M-H]$^-$ ion at *m/z* 283 and base peak at *m/z* 267 amu, in agreement with Han et al. (2007). Compound **34** showed a base peak ion at 495 amu, with a MS^2 fragment at *m/z* 451 and 407 and could not be identified.

3.1.3. *Swartzia polyphylla* (“cumaceba”)

The chemical profile of *S. polyphylla* is presented in Fig. 2c, and the tentative identification of compounds is listed in Table 3. The chromatographic profile shows mainly condensed tannins based on (*epi*)-afzelechin-(*epi*)-catechin, ellagitannins and flavonoids.

Compound **3** presented a base peak ion at *m/z* 353 amu, with a neutral loss of a caffeoyl moiety leading to a MS^2 base peak at *m/z* 191 amu, and a MS^3 fragment at 179 amu. The hierarchical scheme proposed by Clifford et al. (2003) allowed the tentative identification of compound **3** as 5-caffeoylquinic acid.

The compounds **4**, **6–9** and **11, 12** and **14**, differing in the retention times, showed a [M-H]$^-$ base peak at *m/z* 561 and MS^2 fragments at 451, 409 and 289 amu (base peak), in agreement with (*epi*)-afzelechin-(*epi*)-catechin dimers (Sobeh et al., 2018; Lin et al., 2014). The compounds **15**, **16** and **18**, with a [M-H]$^-$ peak at *m/z* 833, differs from the previous flavanol polymers by one additional (*epi*)-afzelechin unit. The compounds were identified as (*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 1, 2 and 3, respectively. Compounds **19** showed a base peak ion at *m/z* 817 amu, with MS^2 fragments at *m/z* 665, 409 and 289 amu, in agreement with cassanidin A (Sobeh et al., 2018). Compound **17** presented a base peak ion at *m/z* 451 amu, with the corresponding MS^2 base peak at *m/z* 341 amu. The fragmentation pattern was in agreement with a phenylpropanoid substituted epicatechin, and was tentatively identified as cinchonain Ia (Bernardo et al., 2018).

Compounds **2** and **5** presented a base peak ion at *m/z* 633, with the loss of 332 amu (galloylhexoside) to the MS^2 main peak at *m/z* 301. Thus, the compounds were tentatively identified as HHDP galloyl hexoside isomers. Compound **1** showed a base peak ion at *m/z* 783, with a neutral loss of 482 amu, in agreement with the loss of a HHDP hexose unit. The compound was tentatively assigned as di-HHDP-hexoside (Pedunculagin) (Fracassetti et al., 2013). Two quercetin derivatives (compounds **20** and **21**) were identified in the sample by the

Table 3
Tentative identification of compounds in the extract from *Swartzia polyphylla* by HPLC-ESI-MS/MS in the negative ion mode.

Peak	Rt (min)	[M-H]$^-$	λ max	MS^2 fragments (% base peak intensity)	Tentative identification
1	6.1	783.6		480.8 (56), 300.7 (100), 256.8 (8)	Di-HHDP-hexoside (Pedunculagin) (Fracassetti et al., 2013)
2	7.8	633.3		300.7 (100)	HHDP galloyl hexoside isomer 1
3	13.6	353.7		190.4 (100), 178.3 (4)	5-caffeoylquinic acid
4	16.5	561.2		543.8 (15), 450.9 (51), 410.1 (61), 288.9 (100)	(*epi*)-azfelechin-(*epi*)-catechin dimer 1
5	17.0	633.7		462.9(42), 300.8(100), 275.0(12), 256.9(2)	HHDP galloyl hexoside isomer 2
6	18.3	561.8		450.9(65), 408.9(100), 289.0(88)	(*epi*)-afzelechin-(*epi*)-catechin dimer 2
7	21.8	561.2		450.9(81), 409.1(83), 289.0(100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 3
8	24.2	561.3		451.0(86), 409.1(85), 288.9(100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 4
9	26.4	561.3		451.0(75), 409.1(71), 288.9(100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 5
10	27.3	447.4		357.0(38), 327.1(100)	Luteolin-*C*-hexoside (Geng et al., 2016)
11	29.0	562.1		451.0(77), 409.0(72), 288.9(100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 4
12	31.0	561.4		451.0(76), 409.1(80), 289.0(100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 5
13	31.6	431.7		340.8 (6), 310.9(100)	Vitexin/Isovitexin (apigenin *C*-hexoside) (Geng et al., 2016)
14	32.0	561.4		451.0(57), 409.1(72), 288.9(100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 6
15	33.0	833.2		681.2(100), 561.6(64), 530.9(73), 289.3(5)	(*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 1
16	36.5	833.2		681.1(67), 561.1(100), 529.1(96), 288.9(14)	(*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 2
17	39.0	451.4		341.0(100)	Cinchonain Ia
18	40.3	833.5		561.3(66), 529.7(100), 289.3(5)	(*epi*)-azfelechin-(*epi*)-azfelechin-(*epi*)-catechin trimer 3
19	42.4	817.6		561.4(100), 289.2(6)	Cassanidin A (Erasto and Majinda, 2003)
20	44.6	477.0		300.8(100)	Quercetin glucuronide
21	47.6	463.6		300.8(100)	Quercetin hexoside
22	51.7	596.8	337sh, 288	434.8(36), 308.8(100), 286.8(62)	Unknown
23	52.6	389.2		371.1 (100), 193.1 (9)	Unknown
24	58.2	271.1		164.4(100)	Dihydroxycoumaric acid hydroxybenzyl ester
25	60.8	270.8	336sh, 288	176.3(23), 150.3(100)	Naringenin
26	62.1	373.1		246.7 (100), 124.3 (65)	Unknown
27	65.2	371.2		303.1(100), 285.8(2)	Prenyl taxifolin
28	67.6	301.0	333sh, 286	124.8(100), 82.1 (100), 56.3(44)	Hesperetin
29	68.7	657.2		481.1(71), 343.2(100)	Hesperetin-*C*-hexoside *O*-glucuronide 1
30	70.7	301.1	333sh, 287	160.4(100)	Unknown
31	71.2	657.2		481.0(71), 343.2(100)	Hesperetin-*C*-hexoside *O*-glucuronide 2
32	74.0	625.3		462.9(100), 325.1(85)	Hesperetin-*C*-hexoside *O*-hexoside
33	77.7	284.9	337sh, 287	269.7(100), 150.5(4)	Hydroxygenistein
34	81.2	355.1	331, 259	228.6(100), 124.5(30)	Unknown

pseudomoleular ions at *m/z* 477 and 463 amu, leading to the base peak at *m/z* 301, indicating the neutral loss of a glucuronic acid and hexose, respectively. Compound **33** showed a base peak ion at *m/z* 285, with a MS^2 ion at *m/z* 269 amu. The compound was tentatively identified as hydroxygenistein (Chang, 2007). The flavone *C*-glycoside luteolin *C*-hexoside (**10**) was identified by the characteristic loss of 120 amu from the parent ion, leading to the base peak at 327 amu, in agreement with the fragmentation pattern described by Geng et al. (2016). The compound **13** was assigned as vitexin/isovitexin *C*-hexoside (apigenin *C*-hexoside).

The compounds **25** and **28** showed UV maxima in agreement with dihydroflavones and $[M-H]^-$ ions at 271 and 301 amu, respectively, differing in one methoxy group and showing similar fragmentation patterns. The mass spectra are in full agreement with naringenin (**25**) and hesperetin (**28**) (Abad-García et al., 2012). The compounds **29** and **31** showed a base peak ion at *m/z* 657, followed by the neutral loss of a glucuronide unit $[M-H-176]^-$ and further loss of 138 amu, leading to the MS^2 base peak ion at *m/z* 343 amu. The compounds were assigned as hesperetin-*C*-hexoside-*O*-glucuronide 1 (**29**) and hesperetin-*C*-hexoside *O*-glucuronide 2 (**31**). Compound **32** was identified as hesperetin-*C*-hexoside-*O*-hexoside, considering the base peak ion at *m/z* 625 and the neutral losses of 162 and 138 amu.

Compound **24** showed a $[M-H]^-$ ion at *m/z* 271 and fragmented to a base peak at *m/z* 164, suggesting the presence of a dihydroxycoumaric acid ester. The compound was tentatively assigned as dihydroxycoumaric acid hydroxybenzyl ester.

Compound **27** showed a $[M-H]^-$ ion at *m/z* 371 amu, and a MS^2 base peak at *m/z* 303 amu. The neutral loss of 68 amu was in agreement with a prenyl chain. The compound was tentatively assigned as prenyl taxifolin. A related compound was previously isolated from *S. polyphylla* (DuBois and Sneden, 1995).

Five peaks were not identified. Compound **22** showed a base peak ion at *m/z* 597 amu, with the neutral loss of a hexoside (-162 amu) and 126 amu, to a MS^2 base peak ion at *m/z* 309. Compound **23** showed a base peak ion at *m/z* 389 amu, and fragments at *m/z* 371 [M-H-18]- and *m/z* 193 $[M-H-196]^-$ amu, the identity remain to be established. Compound **26** and **34** showed base peak ions at *m/z* 373 and 355 amu, respectively, followed by the neutral loss of 126 amu, to a MS^2 base peak ion at *m/z* 247 and 229 amu, respectively. Compound **30** showed a base peak ion at *m/z* 301 amu, to a MS^2 base peak ion at *m/z* 161. The UV profile did not agree with quercetin or ellagic acid, so the identity of the compound remains unassigned.

3.1.4. *Tynanthus panurensis* (“clavo huasca”)

The chemical profile of *T. panurensis* is depicted in Fig. 2d, and the tentative identification of compounds is summarized in Table 4. The HPLC-MS/MS analysis of the sample showed a complex chemical profile. The selected ion chromatogram feature of the Bruker software was used to identify the already reported compounds in *T. panurensis* (Plaza et al., 2005). For the eugenol triglycoside ($C_{26}H_{38}O_{15}$, *m/z* 589), verbascoside and isoverbascoside ($C_{29}H_{36}O_{15}$, *m/z* 623), leucosceptoside ($C_{30}H_{38}O_{15}$, *m/z* 637) and katchimoside ($C_{26}H_{28}O_{14}$, *m/z* 563), the occurrence of the compounds was investigated searching for the $[M-H]^-$ ions. The eugenol triglycoside (compound **27**) was identified by the neutral loss of a pentose and water from the $[M-H]^-$ ion at 589. The compound **41** with a $[M-H]^-$ of 487 amu showed consecutive losses of two hexoses, leading to the *m/z* ion at 163, assigned to eugenol dihexoside. Phenylpropanoids and katchimoside were not detected in our sample.

The mass spectrum of compound **28** shows the loss of 110 amu, leading to the base peak ion at *m/z* 341 amu, and MS^3 ion at *m/z* 217 amu, supporting the identity of the compound as cinchonain Ia or isomer, in agreement with Bernardo et al. (2018). The compound **36** shows the loss of 194 amu, leading to the base peak at 451 amu, and the MS^3 ion at *m/z* 341, suggesting the occurrence of a cinchonain Ia derivative.

The chemical profile of the crude drug shows a rich array of flavone *C*-glycosides, including derivatives of trihydroxyflavone (5,7,4′-trihydroxyflavone, apigenin), tetrahydroxyflavone (5,7,3′,4′-tetrahydroxyflavone, luteolin), pentahydroxyflavone (3,5,7,3′,4′-pentahydroxyflavone, quercetin) and methoxy derivatives. The tentative identification of the compounds was based mainly on the work of Ferreres et al. (2007).

The compounds **17**, **23**, **29**, and **33** shows fragmentation patterns in agreement with apigenin *C*-glycosides (Ferreres et al., 2007). Compound **17** is in agreement with apigenin di *C*-hexoside while **23** is a mono *C*-hexoside (Ferreres et al., 2007). The compounds **29** and **33** shows the loss of 138 and 168 amu, respectively, leading to the base peak at *m/z* 431 amu, which further fragments to *m/z* 311, in agreement with the fragmentation pattern of apigenin *C*-glycosides. The compounds were tentatively identified as apigenin *C*-hexoside derivatives.

The compounds **2**, **5**, **6**, **7**, **10**, **19, 37** and **38** showed the characteristic fragmentation pattern for luteolin *C*-glycoside derivatives. Compound **2** and **6** showed a base peak ions at *m/z* 771, and fragmentations to MS^2 651, 591, 429, 357, 327, in agreement with luteolin *C*-hexoside-*O*-dihexoside isomers 1 and 2 (Ferreres et al., 2007). The compounds **5** and **7** with a $[M-H]^-$ ion at 609 amu fragmented to 357 and 327 amu, in agreement with luteolin di *C*-glycosides (Ferreres et al., 2007). Compound **10** showed a MS base peak ion at *m/z* 593 amu, with a strong MS^2 base peak at *m/z* 473 amu, indicating the loss of a *C*-hexoside. The consecutive loss of [M-H-146]- leading to the MS^2 ion at *m/z* 327 was in agreement with the fragmentation pattern for luteolin-*O*-rhamnoside-*C*-hexoside (Ferreres et al., 2007). Compound **19** was identified as luteolin *C*-hexoside in agreement with literature and UV maxima (Ferreres et al., 2007). The compounds **37** and **38** with $[M-H]^-$ ions at 627 and 597 amu, respectively losses a fragment of 198 and 168 amu, leading to the peak at 429, 357 and base peak at 327, in agreement with luteolin *C*- hexoside derivatives. The compounds were assigned as luteolin *C*-hexoside derivative 1 and 2, respectively.

The compounds **8**, **21** and **22** shows a fragmentation compatible with a monomethoxy derivative of tetrahydroxyflavone (methoxyluteolin), differing in 14 amu units from the corresponding luteolin derivatives. The glycosides **8**, **21** and **22** showed the loss of a hexose, a rhamnose and a pentose and were assigned as methoxyluteolin *C*-hexoside-*O*- hexoside (**8**), methoxyluteolin *C*-hexoside (**21**) and methoxyluteolin *C*-hexoside pentoside (**22**), respectively.

The compounds **4**, **12**, **14**, **15**, **16** and **18** were identified as pentahydroxyflavone *C*-glycosides, all of them presenting the MS^2 ion at *m/z* 343 amu from the pseudomolecular $[M-H]^-$ ions at 625, 463, 595 and 463 amu, respectively. The compounds were assigned as pentahydroxyflavone *C*- dihexoside (**4**), pentahydroxyflavone-*C*-hexoside-*O*-pentoside 1 and 2 (**14** and **15**) and pentahydroxyflavone-*C*-hexoside 1, 2 and 3 (**12**, **16** and **18**). Two additional *C*-glycosides **3** and **11** were tentatively assigned as eriodictyol *C*-glycosides based on the loss of a hexose (162 amu) and 120 amu leading to the base peak at *m/z* 329, in agreement with the fragmentation pattern of eriodictyol *C*-hexosides. The compounds were assigned as eriodictyol *C*-dihexoside (**3**) and eriodictyol-*C*-hexoside (**11**).

The compounds **1** and **13** showed UV spectra compatible with caffeic acid derivatives. Compound **1** presented a base peak ion at *m/z* 353 amu, followed by the neutral loss of a caffeoyl moiety to a MS^2 base peak at *m/z* 191 amu, and a MS^3 fragment at 179 amu. The hierarchical scheme proposed by Clifford et al. (2003) allowed the tentative identification of compound **1** as 5-caffeoylquinic acid. Compound **13** was tentatively assigned as caffeoyl shikimic acid due to the base peak at *m/z* 335 with MS^2 ions at *m/z* 179, 161 and 135 amu (Ben Said et al., 2017). Compound **9** presented a base peak ion at *m/z* 337 amu, with secondary ions at MS^2 191 and 163, in agreement with 5-*p*-coumaroylquinic acid (Clifford et al., 2003).

The compounds **24**, **25**, **30, 31, 32** and **35** were assigned as flavonoid *O*-glycosides. Compounds **24**, **25, 32** and **35** showed a common

Table 4
Tentative identification of compounds in the extract from *Thynanthus panurensis* by HPLC-ESI-MS/MS in the negative ion mode.

Peak	Rt (min)	[M-H]$^-$	λ max	MS^2 fragments (% base peak intensity)	Tentative identification
1	13.5	353.1	325, 292sh	190.5 (100), 179.1 (5)	5-caffeoylquinic acid
2	14.3	771.2		651.1 (8), 357.0(32), 327.0(100)	Luteolin-*C*-hexoside-*O*-dihexoside isomer 1
3	14.5	611.4		328.9 (100), 192.5 (5)	Eriodictyol *C*-dihexoside
4	15.8	625.4		342.9(100), 206.9(5)	Pentahydroxyflavone *C*-dihexoside
5	17.2	609.4	348, 268	357.0(63), 327.6(100)	Luteolin *C*-dihexoside 1
6	17.3	771.5		591.1 (5), 429.2 (8) 357.2(59), 327.5(100)	Luteolin *C*-hexoside -*O*-dihexoside isomer 2
7	19.8	609.3	348,271	357.0(65), 327.2(100)	Luteolin *C*-dihexoside 2
8	20.1	623.5		461.1(52), 341.5(100)	Methoxyluteolin-*C*-hexoside-*O*-hexoside
9	20.5	337.5		191.0(100), 162.4(7)	5-*p*-Coumaroylquinic acid
10	20.8	593.5		473.2(100), 327.6(7)	Luteolin-*O*-rhamnoside-*C*-hexoside (Ferreres et al., 2007)
11	21.2	449.6		328.9(100)	Eriodictyol-*C*-hexoside (Stalmach et al., 2009)
12	21.4	463.6		372.9(9), 343.0(100)	Pentahydroxyflavone-*C*-hexoside 1
13	21.8	335.7	324,293sh	178.5(100)	Caffeoyl shikimic acid (Ben Said et al., 2017)
14	22.3	595.5		475.1(100), 343.7(5)	Pentahydroxyflavone-*C*-hexoside-*O*-pentoside 1
15	24.6	595.4		475.1(100), 343.6(6)	Pentahydroxyflavone -*C*-hexoside-*O*-pentoside 2
16	24.9	463.3		342.9(100)	Pentahydroxyflavone-*C*-hexoside 2
17	25.1	593.5		311.1(100)	Apigenin *C*- dihexoside
18	25.2	463.4		372.9(7), 342.9(100)	Pentahydroxyflavone-*C*-hexoside 3
19	27.4	447.2	348, 268	356.9(41), 327.1(100)	Luteolin *C*-hexoside
20	28.2	579.7		459.0(100), 429.1(12), 357.6(9)	Unknown
21	28.8	461.3		341.0(100), 313.5(38)	Methoxyluteolin *C*-hexoside
22	29.1	593.4	348, 271	443.2(100), 343.5(16)	Methoxyluteolin *C*-hexoside pentoside
23	34.7	431.6	340, 237	311.0(100)	Apigenin *C*-hexoside
24	34.9	609.5		300.8 (100)	Quercetin rutinoside
25	35.0	447.1		300.9 (100)	Quercetin rhamnoside
26	35.8	577.4	341, 236	427.2(100), 327.9(5)	Unknown
27	37.4	589.4	338, 237	424.9 (100), 323.1 (22)	Eugenol dipentoside hexoside
28	39.2	451.3		340.9 (100); 341.8–216.9(100)	Cichonain Ia or isomer
29	42.7	569.5		430.9(100), 310.9(31)	Apigenin *C*-hexoside derivative 1
30	43.8	447.9		314.9(100)	Isorhamnetin pentoside
31	44.8	447.6		284.7(100)	Kaempferol/luteolin hexoside
32	45.1	477.6		300.8	Quercetin glucuronide
33	45.5	599.8		430.9(100), 311.1(14)	Apigenin *C*-hexoside derivative 2
34	46.0	597.4	348, 267	428.9(100), 308.9(7)	Unknown
35	47.6	463.5		300.8(100)	Quercetin hexoside
36	49.6	645.7		451.2(100), 341.3(33)	Cinchonain Ia derivative
37	52.4	627.4		428.9(12), 357.0(29), 326.9(100)	Luteolin *C*-hexoside derivative 1
38	53.3	597.3	347, 271	428.9(8), 357.0(17), 327.0(100)	Luteolin *C*-hexoside derivative 2
39	60.2	719.7		580.7 (100), 536.7 (53), 398.8 (48)	Unknown
40	66.3	779.5		595.1 (93), 508.9 (61)	Unknown
41	75.8	487.9		324.99(100) , 163	Eugenol dihexoside

base peak ion at MS *m/z* 301 amu and a UV profile in agreement with quercetin. The loss of −308 amu (rutin), −146 (rhamnose), −176 (glucuronide) and −162 (hexose), allowed the tentative identification of these compounds as quercetin rutinoside (**24**), quercetin rhamnoside (**25**), quercetin glucuronide (**32**) and quercetin hexoside (**35**). Compound **30** showed a base peak ion at *m/z* 447 amu, leading to a MS^2 ion at 315 amu, and was tentatively identified as isorhamnetin pentoside. Compound **31** showed a base peak ion at *m/z* 447, leading to a MS^2 ion at 285. The UV spectrum did not allow the differentiation of this compound, and thus was assigned as kaempferol/luteolin hexoside.

The compounds **26** and **34** show a fragmentation pattern compatible with *C*-glycosides. Moreover, the UV spectrum presents UV maxima in agreement with a flavone *C*-glycoside. However, it was not possible to assign the structure. In addition, the identity of compounds **20**, **39** and **40** was not clear.

3.2. Chemical profiles of the traditional preparations LL and SVSS

The HPLC-DAD-ESI-MS profiles of the traditional preparations, LL and SVSS are shown in Fig. 3. The identity of compounds identified in both preparations is presented in Table 5. The presence of the different ingredients was established following the matching in the retention time and MS fragmentation of the constituents identified in the single crude drug profiles.

For LL, 39 peaks were detected in the Total Ion Chromatogram (TIC, Fig. 3a, gray). The traditional preparation of LL is a mixture of *S. polyphylla*, *T. panurensis* and *M. guianensis,* bark in a 1:1:1 ratio, approximately. The profile shows some resemblance to that of *S. polyphylla* (Rt 50–75 min). Table 5 shows that 16 compounds from this plant were present in the mixture, including 5-caffeoylquinic acid (**1**), (*epi*)-afzelechin-(*epi*)-catechin dimers and trimers (peaks **5**, **21** and **37**), *C*-glycosides of flavonoids (peaks **23** and **29**), dihydroxycoumaric acid hydroxylbenzyl ester (**54**), naringenin (**56**), prenyl taxifolin (**58**), hesperetin (**59**) and hydroxygenistein (**64**). In addition, 16 signals belonging to *T. panurensis* were detected, most of them were in low intensity in the UV chromatogram. Of these compounds, most were *C*-glycoside derivatives (peaks **2**-**4**, **8**-**10**, **12**, **14**, **18**, **24**, **25**, **52** and **53**). For *M. guianensis* only 5 signals were detected in the LL preparation, mainly based in (*epi*)-afzelechin-(*epi*)-catechin dimers and trimers (peaks **11**, **16**, **28** and **35**). Some 7 other signals were detected in the mixture that were not detected in any of the declared ingredients. The compounds **32**, **36**, **43**, **48**, **50**, **51**, **55, 62** and **65** were not identified, and of those, only compounds **50** and **62** were intense peaks in the UV chromatogram (Fig. 3a, black).

The traditional preparation SVSS is a mixture of *S. polyphylla*, *T. panurensis* and *C. angustifolia.* In a similar way to LL, the UV profile of SVSS shows a very similar pattern to that of *S. polyphylla* between Rt 50–75 min (Fig. 3b). In this preparation, a total of 57 peaks of the TIC were detected and identified. Of those, 18 peaks can be attributed to *S. polyphylla*, 21 peaks from *T. panurensis* and 19 peaks from *C. angustifolia.* The peaks from *S. polyphylla* are mainly based of *epi*-afzelechin-(*epi*)-catechin dimers (peaks **11**, **13**, **21**, **30**, and **38**), while the peaks

Table 5
Tentative identification of constituents in the extracts from the traditional Peruvian preparations LL and SVSS by HPLC-ESI-MS/MS.

Peak	Rt (min)	[M-H]$^-$	MS^2 fragments (% base peak intensity)	Tentative identification	Detected in		Origin
					LL	SVSS	(peak number)[a]
1	13.8	353.5	190.5 (100), 178.40 (13)	5-caffeoylquinic acid	x	x	Ca (1), Sp (3), Tp (1)
2	14.7	771.2	357.0(32), 327.0(100)	Luteolin *C*-hexoside-*O*-dihexoside 1	x	x	Tp (2)
3	15.2–15.9	625.4	342.9 (100), 206.6 (5)	Pentahydroxyflavone *C*-dihexoside 1	x	x	Tp (4)
4	17.3–17.5	771.3	359.9(53), 327.0(100)	Luteolin *C*-hexoside-*O*-dihexoside 2	x	x	Tp (6)
5	18.7	561.7	451.0 (61), 408.9 (87), 288.8 (57)	(*epi*)-afzelechin-(*epi*)-catechin dimer 1	x		Sp (6)
6	18.8	561.5	451.0 (69), 409.4 (98), 289.1 (98)	(*epi*)-afzelechin-(*epi*)-catechin dimer 2		x	Ca (5)
7	20.2	610.1	411.0(28), 357.0(74), 327.2(100)	Luteolin *C*-dihexoside 1		x	Tp (7)
8	20.5	623.1	461.1 (42), 341.3 (100)	Methoxyluteolin *C*-hexoside-*O*-hexoside 1	x	x	Tp (8)
9	20.9	593.4	503.0(19), 473.2(100), 327.3(14)	Luteolin-*O*-rhamnoside-*C*-hexoside	x	x	Tp (10)
10	21.5	463.6	372.9(9), 343.0(100)	Pentahydroxyflavone *C*-hexoside 1	x	x	Tp (12)
11	21.9	561.1	450.9 (86), 409.1 (78), 288.9 (95)	(*epi*)-afzelechin-(*epi*)-catechin dimer 3	x	x	Ca (6), Mg (12)
12	22.4	595.1	475.1 (100)	Pentahydroxyflavone *C*-hexoside-*O*-pentoside 1	x	x	Tp (14)
13	23.3	561.4	450.9(92), 409.0(100), 288.9(97)	(*epi*)-azfelechin-(*epi*)-catechin dimer 4		x	Sp (8)
14	24.6	595.4	475.1(100), 343.6(6)	Pentahydroxyflavone *C*-hexoside-*O*-pentoside 2	x	x	Tp (15)
15	24.9	561.2	408.9 (83), 288.9 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 5		x	Ca (7)
16	25.0	561.5	451.0 (63), 409.0 (28), 288.8 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 6	x		Mg (16)
17	25.1	561.3	409.1 (97), 288.9 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 7		x	Ca (8)
18	25.2	463.4	372.9(7), 342.9(100)	Pentahydroxyflavone *C*-hexoside 2	x	x	Tp (16)
19	25.6	593.1	311.1 (100)	Apigenin *C*-dihexoside		x	Tp (17)
20	26.5	561.3	409.1 (100), 288.9 (79)	(*epi*)-afzelechin-(*epi*)-catechin dimer 8		x	Ca (10)
21	26.8–27.2	561.5	451.0 (98), 409.0 (85), 288.8 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 9	x	x	Sp (9)
22	27.3	545.7	435.1 (39), 409.1 (34) 288.9 (100)	(*epi*)-guibourtinidol-(*epi*)-catechin dimer		x	Ca (11)
23	27.6	447.2	357.0(37), 327.1(100)	Luteolin-*C*-hexoside	x	x	Sp (10), Tp (19)
24	29.0	461.5	341.7(100), 313.1(25)	Methoxyluteolin *C*-hexoside	x	x	Tp (21)
25	29.5	593.4	443.2(100), 343.4(20	Methoxyluteolin *C*-hexoside pentoside 1	x	x	Tp (22)
26	29.9	579.7	459.0(100), 429.1(24), 309.1(29)	Unknown		x	–
27	31.0	593.5	443.5(84), 343.9(62)	Methoxyluteolin *C*-hexoside pentoside 2		x	–
28	31.4–31.7	561.1	451.0 (66), 409.0 (69), 288.8 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 10	x		Mg (21), Sp (12)
29	31.9	431.2	310.9(100)	Apigenin *C*-hexoside	x	x	Tp (23) Sp (13)
30	32.5	561.5	451.0(5), 409.0 (21), 288.9(100)	(*epi*)-azfelechin-(*epi*)-catechin dimer 11		x	Sp (14)
31	33.4	575.2	422.8 (100), 406.9 (34), 288.7 (15)	A-type procyanidin dimer		x	Ca (14)
32	34.9–36.1	577.5	457.0(100), 327.2(54)	Unknown	x	x	Tp (26)
33	35.7	833.3	681.3 (60), 529.9 (100), 289.3 (8)	(*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 1		x	Ca (17)
34	36.4	561.4	451.0 (82), 408.9 (86), 288.8 (100)	(*epi*)-afzelechin-(*epi*)-catechin dimer 12		x	Ca (18)
35	36.5	833.2	681.2 (60), 561.1 (86), 529.1 (100), 288.9 (7)	(*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 2	x		Mg (26), Sp (16)
36	38.5	607.6	457.2 (100), 357.5 (5)	Unknown	x	x	–
37	39.5–40.6	833.3	681.2 (100),561.2 (67), 529.9 (55), 289.4 (5)	(*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 3		x	Ca (20)
38	42.2	833.2	681.1 (41), 561.2 (85), 529.9 (68)	(*epi*)-azfelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 4	x	x	Sp (18)
39	42.8	817.6	561.3 (100), 409.3 (8), 289.3 (3)	Cassanidin A isomer 1		x	Sp (19)
40	43.3	711.7	561.1 (100), 543.2 (53), 391.2 (12), 271.0 (14)	Galloyl-(*epi*)-afzelechin-(*epi*)-catechin isomer 1		x	Ca (23)
41	43.9	817.6	561.3 (100), 409.3 (8), 289.3 (3)	Cassanidin A isomer 2		x	Ca (24)
42	45.7	599.8	445.0(100), 430.9 (95), 325.6(79),	Apigenin *C*-hexoside derivative		x	Tp (33)
43	45.8	583.4	444.9 (100), 325.1 (21)	Unknown	x		–
44	46.8	597.3	428.9 (100), 327.0 (7)	Unknown		x	Tp (34)
45	47.1	817.5	665.0 (58), 561.1 (100), 289.0 (5)	(*epi*)-guibourtinidol-(*epi*)-afzelechin-(*epi*)-catechin trimer	x		Mg (31)
46	47.4	833.6	681.2 (100), 561.6 (64), 530.9 (73), 289.3 (5)	(*epi*)-afzelechin-(*epi*)-afzelechin-(*epi*)-catechin trimer 5		x	Ca (25)
47	49.2	712.9	561.0 (100), 543.0 (43), 271.0 (14)	Galloyl-(*epi*)-afzelechin-(*epi*)-catechin isomer 2		x	Ca (26)
48	50.8	583.3	445.0 (100), 325.1 (19)	Unknown	x		–
49	51.7	801.2	765 (6), 691.0 (54), 561.1 (100), 545,2 (90), 409.2 (28), 288.9 (25)	(*epi*)-guibourtinidol-(*epi*)-guibourtinidol-(*epi*)-catechin trimer		x	Ca (27)
50	52.3	583.3	445.0 (100), 325.1 (19)	Unknown	x	x	–
51	52.6	389.2	371.1 (100), 193.1 (9)	Unknown	x	x	Sp (22)
52	52.7	627.2	428.9 (11), 356.9 (22), 326.9 (68)	Luteolin *C*-hexoside derivative 1	x		Tp (37)
53	53.5	597.7	428.9 (17), 326.9 (100)	Luteolin *C*-hexoside derivative 2	x	x	Tp (38)
54	58.7	271.7	164.3 (100)	Dihydroxycoumaric acid hydroxybenzyl ester	x	x	Sp (24)
55	60.1	557.4	412.9 (100)	Unknown	x		–

(*continued on next page*)

Table 5 (*continued*)

Peak	Rt (min)	[M-H]⁻	MS^2 fragments (% base peak intensity)	Tentative identification	Detected in		Origin
					LL	SVSS	(peak number)[a]
56	61.1	271.2	176.3 (27), 160.4 (8) 150.3 (100)	Naringenin	x	x	Sp (25), Ca (28)
57	62.3–62.6	373.5	246.7 (100), 124.4 (88)	Unknown	x	x	Sp (26)
58	65.5	371.4	303.1(100)	Prenyl taxifolin	x	x	Sp (27)
59	68.5	301.0	124.2(100)	Hesperetin	x	x	Sp (28)
60	68.6	495.4	451.1 (100), 407.9 (17)	Unknown		x	Ca (29)
61	69.1	657.7	480.9 (44), 343.0 (100)	Hesperetin *C*-hexoside-*O*-glucuronide		x	Sp (29)
62	70.2–71.2	299.1	283.7(45), 230.7(100)	Methoxy trihydroxy flavanone	x	x	Sp (30)
63	72.4	389.7	778.9 [2M-H], 371.1 (100), 307.1 (44)	Unknown		x	–
64	77.8–78.0	285.0	269.7(100), 163.8(2), 150.5(3)	Hydroxygenistein	x	x	Sp (33), Ca (30)
65	81.4	355.3	228.6 (70), 124.5 (40)	Unknown	x		Sp (34)
66	82.0	392.5	783.5 [2M-H], 373.3 (100)	Unknown		x	–
67	84.0	353.5	309.0 (7), 284.7 (100)	Unknown		x	–

[a] Ca: Campsiandra angustifolia; Mg: Minquartia guianensis; Sp: Swartzia polyphylla, Tp: Tynanthus panurensis. The numbers in parenthesis are related to Tables 1–4 for each species, respectively.

from *T. panurensis* are mainly *C*-glycosides (peaks **2**-**4**, **7**-**10**, **12**, **14**, **18**, **19**, **23**-**25**, **27**, **29**, **42** and **53**). For *C. angustifolia*, (*epi*)-afzelechin-(*epi*)-catechin dimers and trimers (peaks **6**, **15**, **17**, **20**, **33**, **34**, **37**, **40**, **46** and **47**) were the main constituents present in SVSS. Four compounds (peaks **26**, **36**, **64** and **67**) were present in the mixture that were not detected in any of the declared ingredients. The identity of these five compounds was not clear from the UV profile or MS fragmentation, and remains to be established.

Under our experimental conditions, searching for pro-sexual drugs, Sildenafil® showed a Rt of 9.66 min, with UV profile showing maximum at 295 nm. The extracted ion chromatogram of sildenafil, vardenafil, tadalafil, homosildenafil, hydroxyhomosildenafil, piperidino sildenafil, aildenafil, thiosildenafil, thiohomosildenafil, thioaildenafil, acetildenafil, noracetildenafil, hydroxyacetildenafil, piperidinoacetildenafil, piperidenafil, aminotadalafil and benzamidenafil showed that none of them occurs in the samples.

3.3. Inhibitory activity towards PDE-5

The inhibition of the enzyme PDE-5 is the molecular mechanism of action responsible for the effect of the commercial drug sildenafil citrate (Viagra®). The extracts from the single ingredients as well as from the traditional preparations were assessed at 100 and 200 µg/mL (Table 6). At 200 and 100 µg/mL, the extract from the preparation LL and SVSS inhibited the PDE-5 by 49.88% and 27.90% and 42.39% and 12.31%, respectively. The extracts from the single constituents showed strong differences, ranging from inactive for *M. guianensis* to low effect for *S. polyphylla* and *T. panurensis* and high activity for *C. angustifolia* which inhibited the enzyme by 89.37% and 81.32% at 200 and 100 µg/mL, respectively (Table 6). Under the same experimental conditions, the IC_{50} of the reference drug sildenafil was 21.07 ± 0.61 µg/mL. The ingredient of the traditional preparation most active towards the PDE-5 was *Campsiandra angustifolia* with some contribution of *T. panurensis*.

Table 6
Inhibitory effect towards the enzyme phosphodiesterase-5 (PDE-5) of the extracts from the traditional Peruvian Amazon preparations LL and SVSS and their crude drug constituents.

Sample	%Inhibition of PDE-5	
	200 µg/mL	100 µg/mL
Traditional preparation		
LL	49.88 ± 0.64	27.90 ± 4.52
SVSS	42.39 ± 2.91	12.31 ± 3.99
Crude drugs		
Campsiandra angustifolia	89.37 ± 0.65	81.32 ± 2.23
Minquartia guianensis	Inactive	Inactive
Swartzia polyphylla	21.03 ± 1.90	13.82 ± 1.98
Tynanthus panurensis	28.10 ± 3.82	22.96 ± 1.99
Reference compound	IC_{50} (µg/mL)	
Sildenafil citrate	21.07 ± 0.61	

4. Discussion

Campsiandra angustifolia Spruce ex Benth (Fabaceae) is a tree growing in the tropical forests of the Peruvian Amazon. The traditional use found in literature is for the bark or the fruit, which are macerated to treat rheumatism, joint pain, fever, diarrhea and liver illnesses (Vásquez-Ocmín et al., 2018). The chemical profile of *C. angustifolia* (Fig. 2a) shows mainly proanthocyanidins and gallotannins, flavonoids and caffeoylquinic acid (Table 1). *Minquartia guianensis* has been reported to contain xanthones, oleanolic acid derivatives, and acetylenic compounds with cytotoxic and antiprotozoal activities (Marles et al., 1989; El-Seedi et al., 1994). The chemical profile (Fig. 2b) shows condensed and hydrolyzable tannins and flavonoids. Previous studies on *Swartzia polyphylla* were performed on the heartwood of the tree, searching for bioactive compounds against cariogenic bacteria (Osawa et al., 1992). The authors reported that the investigation was carried out with the wood purchased at a Peruvian market but additional information was not provided. Several isoflavanones were isolated and the structures were determined by spectroscopic and spectrometric methods, confirming the occurrence of naringenin, formononetin, bioachanin A, dihydrobioachanin A, ferreirin, dihydrocajanin and darbergioidin (Osawa et al., 1992). Diterpenes from the cassane skeleton were isolated from the dichloromethane extract of the roots from *Swartzia simplex* from Panama (Favre-Godal et al., 2015). In our work (Fig. 2c) condensed tannins and flavonoids were the main constituents. A previous study on *Tynanthus panurensis*, using a sample from the Peruvian Ucayali, afforded a new eugenol triglycoside, the known phenylpropanoid glycosides verbascoside, isoverbascoside, leucosceptoside and the apigenin *C*-glycoside katchimoside (Plaza et al., 2005). The compounds were isolated from the MeOH extract of the tree bark and the structures were unambiguously determined by spectroscopic and spectrometric means. In our sample (Fig. 2d), flavonoid *C*-glycosides and *O*-glycosides as well as quinic acid derivatives were identified.

Previous surveys on medicinal plants of the Peruvian Amazon are mainly focused on the search for antiprotozoal crude drugs (Kvist et al., 2006; Ruiz et al., 2011; Odonne et al., 2013; Vásquez-Ocmín et al., 2018) or plants used in shamanic initiation (Jauregui et al., 2011). Two of the species used to prepare the traditional sexual enhancers from the

Iquitos market, *M. guianensis* and *T. panurensis*, are mentioned as "plant teachers" in Jauregui et al. (2011). The bark and trunk decoction of *T. panurensis* is orally taken for strengthening in the teaching process for shamanic initiation (Jauregui et al., 2011). According to Odonne et al. (2013), the bark of *T. panurensis* is used to treat anemia and sterility. The bark is macerated in sugarcane alcohol and taken as a tonic. The Fabaceae *Swartzia myrtifolia* Sm. and *S. simplex* (Sw.) Spreng roots are grated in water and orally taken as a tonic, while the bark decoction of *M. guianensis* is orally taken and vomited to treat malaria (Odonne et al., 2013). *Campsiandra angustifolia* and *M. guianensis* are used in the Loreto area (Peru) for rheumatism and malaria (febrifuge). The crude drug is prepared by macerating 100 g of the bark of *C. angustifolia* or 200 g of *M. guianensis* bark in 1 L of sugar cane distillate. One cup is orally taken with an empty stomach for a month (Vásquez-Ocmín et al., 2018). *Campsiandra angustifolia* and *M. guianensis* are mentioned by Kvist et al. (2006) and by Ruiz et al. (2011) as species used to treat malaria in the Nanay river area, close to Iquitos, Peru. Both plants present antiplasmodial activity (Ruiz et al., 2011).

The chemical profiles of the traditional preparations LL and SVSS showed consistency with the declared ingredients (Fig. 3). The main compounds present in the traditional preparations resemble the profile of *Swartzia polyphylla* and *T. panurensis*. However, in both products we found signals for compounds that were not detected in the declared ingredients, including compounds whose identities could not be established. The manufacture of herbal remedies is not properly regulated and the presence of signals that do not match with the profiles of the declared ingredients could be an indicator of the use of other materials in the traditional preparation or results of the natural variability of the single ingredients. The use of unlisted ingredients in herbal remedies has been reported in the past (Ching et al., 2018). For example, Bogusz et al. (2006) found the presence of sildenafil and tadalafil in supposedly "pure herbal" remedies, as well as the presence of glibenclamide in herbal products to treat diabetes. A study published in 2015 showed that 61% from a total of 150 supplements were altered with synthetic PDE-5 inhibitors (Gilard et al., 2015). The use of adulterants in herbal remedies may be potentially dangerous and cause serious health damages. The identity of these unidentified signals in our samples remains to be established.

Under our experimental conditions, *C. angustifolia*, *T. panurensis* and the traditional preparations LL and SVSS inhibited PDE-5 at 200 and 100 μg/mL (Table 6). Some secondary metabolites have been recognized as active inhibitors of PDE-5. For example, 8-prenylkaempferol-3,7-diglucoside (icariin), is a potent inhibitor of human PDE-5 (Dell'Agli et al., 2008). Our results show that this compound is not present in the active ingredients or in the traditional preparations. Other male aphrodisiacs used traditionally in Peru are tubers and roots from *Corynaea crassa* Hook. F. (Balanophoraceae). The constituents identified in the plant are mainly known triterpene and sterols (Garcia et al., 2015). Tang et al. (2017) showed that a seed extract from *Allium tuberosum* (Amaryllidaceae), used as a male aphrodisiac in China, had the ability to significantly relax an isolated corpus cavernosum smooth muscle from rats in a concentration dependent manner. In addition, the extract was able to increase the mount frequency, intromission frequency and intromission latency. The secondary metabolites identified in this extract were mainly steroidal saponins and nucleosides.

5. Conclusions

The present study reports for the first time the chemical composition of the crude drugs *C. angustifolia*, *M. guianensis*, *S. polyphylla* and *T. panurensis*, used to prepare male sexual enhancers in the Peruvian Amazon. The traditional preparations LL and SVSS were analyzed and the composition was compared with that of the single constituents. A total of 31 compounds were detected in *C. angustifolia* and *M. guianensis*, 34 compounds for *S. polyphylla* and 41 compounds for *T. panurensis*. In the preparations LL and SVSS, some 67 compounds were tentatively identified. The main components were condensed and hydrolysable tannins, as well as *C*-glycosyl flavones. Under our assay conditions, the *C. angustifolia* extract was a potent inhibitor of PDE-5, followed by *S. polyphylla* and *T. panurensis*. LL and SVSS also inhibited PDE-5. Adulterants were not detected, thus indicating that the inhibition of PDE-5 is due to the chemical components of the crude drugs and traditional preparations. Some compounds present in the traditional preparation were not detected in the declared ingredients. The collection places, harvesting time and storage conditions of the crude drugs used for the beverages is unknown. Therefore, further work should be carried out to establish the natural variability and content of the single constituents. The present study sets the basis for further investigations on these crude drugs and preparations, including the isolation of main components for the complete chemical characterization, as wells as the bioactive guided fractionation and isolation of the compounds responsible for the inhibition of PDE-5.

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Author's contribution

Schmeda-Hirschmann, Jiménez-Aspee, Theoduloz designed the research. The field work was carried out by Vargas-Arana and Schmeda-Hirschmann. Jiménez-Aspee, Theoduloz, Burgos-Edwards conducted the experiments. All authors contributed to the data analysis and writing of the manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest associated with this manuscript.

References

Abad-García, B., Garmón-Lobato, S., Berrueta, L.A., Gallo, B., Vicente, F., 2012. On line characterization of 58 phenolic compounds in *Citrus* fruit juices from Spanish cultivars by high-performance liquid chromatography with photodiode-array detection coupled to electrospray ionization triple quadrupole mass spectrometry. Talanta 99, 213–224.

Abusnina, A., Lugnier, C., 2017. Therapeutics potentials of natural compounds acting on cyclic nucleotide phosphodiesterase families. Cell Sign. 39, 55–65.

Beharry, S., Heinrich, M., 2018. Is the hype around the reproductive health claims of maca (*Lepidium meyenii* Walp.) justified? J. Ethnopharmacol. 211, 126–170.

Ben Said, R., Hamed, A.I., Mahalel, U.A., Al-Ayed, A.S., Kowalczyk, M., Moldoch, J., Oleszek, W., Stochmal, A., 2017. Tentative characterization of polyphenolic compounds in the male flowers of *Phoenix dactylifera* by liquid chromatography coupled with mass spectrometry and DFT. Int. J. Mol. Sci. 18, 512.

Bernardo, J., Ferreres, F., Gil-Izquierdo, A., Videira, R.M., Valentao, P., Veiga, F., Andrade, P.B., 2018. In vitro multimodal-effect of *Trichilia catigua* A. Juss. (Meliaceae) bark aqueous extract in CNS targets. J. Ethnopharmacol. 211, 247–255.

Bischoff, E., 2004. Potency, selectivity, and consequences of nonselectivity of PDE inhibition. Int. J. Impot. Res. 16, S11–S14.

Bogusz, M.J., Hassan, H., Al-Enazi, E., Ibrahim, Z., Al-Tufail, M., 2006. Application of LC-ESI-MS-MS for detection of synthetic adulterants in herbal remedies. J. Pharm. Biomed. Anal. 41, 554–564.

Ching, C.K., Chen, S.P.L., Lee, H.H.C., Lam, Y.H., Ng, S.W., Chen, M.L., Tang, M.H.Y., Chan, S.S.S., Ng, C.W.Y., Cheung, J.W.L., Chan, T.Y.C., Lau, N.K.C., Chong, Y.K., Mak, T.W.L., 2018. Adulteration of proprietary Chinese medicines and health products with undeclared drugs: experience of a tertiary toxicology laboratory in Hong Kong. Br. J. Clin. Pharmacol. 84, 172–178.

Clifford, M.N., Johnston, K.L., Knight, S., Kuhnert, N., 2003. Hierarchical scheme for LC-MS^n identification of chlorogenic acids. J. Agric. Food Chem. 51, 2900–2911.

Corbin, J.D., Francis, S.H., 2003. Molecular biology and pharmacology of PDE-5-inhibitor therapy for erectile dysfunction. J. Androl. 24, S38–S41.

Dell'Agli, M., Galli, G.V., Dal Cero, E., Belluti, F., Matera, R., Zironi, E., Pagliuca, G., Bosisio, E., 2008. Potent inhibition of human phosphodiesterase-5 by Icariin derivatives. J. Nat. Prod. 71, 1513–1517.

Drewes, S.E., George, J., Khan, F., 2003. Recent findings on natural products with erectile-dysfunction activity. Phytochemistry 62, 1019–1025.
DuBois, J.L., Sneden, A.T., 1995. Dihydrolicoisoflavone, a new isoflavanone from *Swartzia polyphylla*. J. Nat. Prod. 58, 629–632.
El-Seedi, H.R., Hazell, A.C., Torssell, K.B.G., 1994. Triterpenes, lichexanthone and an acetylenic acid from *Minquartia guianensis*. Phytochemistry 35, 1297–1299.
Erasto, P., Majinda, R., 2003. Bioactive proanthocyanidins from the root bark of *Cassia abbreviata*. Int. J. Biol. Chem. Sci. 5, 2170–2179.
Favre-Godal, Q., Dorsaz, S., Queiroz, E.F., Marcourt, L., Ebrahimi, S.N., Allard, P.M., Voinesco, F., Hamburger, M., Gupta, M.P., Gindro, K., Sanglard, D., Wolfender, J.L., 2015. Anti-*Candida* cassane-type diterpenoids from the root bark of *Swartzia simplex*. J. Nat. Prod. 78, 2994–3004.
Fariña-Pérez, L.A., Meijide-Rico, F., 2015. Propiedades de la manteca de bufeo, del chuchuhuasi, el cocobolo, la clabohuasca, la huacapuruna, el ipururo y el viborachado: afrodisiacos populares peruanos en Pantaleón y las visitadoras, de Mario Vargas Llosa. Rev. Int. Androl. 13, 113–114.
Ferreres, F., Gil-Izquierdo, A., Andrade, P.B., Valentão, P., Tomás-Barberán, F.A., 2007. Characterization of *C*-glycosyl flavones *O*-glycosilated by liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1161, 214–223.
Fracassetti, D., Costa, C., Moulay, L., Tomás-Barberán, F.A., 2013. Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (*Myrciaria dubia*). Food Chem. 139 (578-558).
Garcia, G.R.M., Lothar Hennig, L., Joachim Sieler, J., Bussmann, R.W., 2015. Constituents of *Corynaea crassa* "Peruvian Viagra". Rev. Bras. Farmacogn. 25, 92–97.
Geng, P., Sun, J., Zhang, M., Li, X., Harnly, J.M., Chen, P., 2016. Comprehensive characterization of *C*-glycosyl flavones in wheat (*Triticum aestivum* L.) germ using UPLC-PDA-ESI/HRMS and mass defect filtering. J. Mass Spectrom. 51, 914–930.
Gilard, V., Balayssac, S., Tinaugus, A., Martins, N., Martino, R., Malet-Martino, M., 2015. Detection, identification and quantification by ^{1}H NMR of adulterants in 150 herbal dietary supplements marketed for improving sexual performance. J. Pharm. Biomed. Anal. 102, 476–493.
Han, J., Ye, M., Xu, M., Sun, J., Wang, B., Guo, D., 2007. Characterization of flavonoids in the traditional Chinese herbal medicine-Huangqin by liquid chromatography coupled with electrospray ionization mass spectrometry. J. Chromatogr. B 848, 355–362.
Jauregui, X., Clavo, Z.M., Jovel, E.M., Pardo-de-Santayana, M., 2011. "Plantas con madre": plants that teach and guide in the shamanic initiation process in the East-Central Peruvian Amazon. J. Ethnopharmacol. 134, 739–752.
Kvist, L.P., Christensen, S.B., Rasmussen, H.B., Mejia, K., Gonzalez, A., 2006. Identification and evaluation of Peruvian plants used to treat malaria and leishmaniasis. J. Ethnopharmacol. 106, 390–402.
Lin, L.Z., Sun, J., Chen, P., Monagas, M.J., Harnly, J.M., 2014. UHPLC-PDA-ESI/HRMS^{n} profiling method to identify and quantify oligomeric proanthocyanidins in plant products. J. Agric. Food Chem. 62, 9387–9400.
Marles, R.J., Farnsworth, N.R., Neill, D.A., 1989. Isolation of a novel cytotoxic polyacetylene from a traditional anthelmintic medicinal plant *Minquartia guianensis*. J. Nat. Prod. 52, 261–266.
Melnyk, J.P., Marcone, M.F., 2011. Aphrodisiacs from plant and animal sources – a review of current scientific literature. Food Res. Int. 44, 840–850.
Molina-Calle, M., Priego-Capote, F., Luque de Castro, M.D., 2015. Development and application of a quantitative method for determination of flavonoids in orange peel: influence of sample pretreatment on composition. Talanta 144, 349–355.
Odonne, G., Valadeau, C., Alban-Castillo, J., Stien, D., Sauvain, M., Bourdy, G., 2013. Medical ethnobotany of the Chayahuita of the Paranapura basin (Peruvian Amazon). J. Ethnopharmacol. 146, 127–153.
Osawa, K., Yasuda, H., Maruyama, T., Morita, H., Takeya, K., Itokawa, H., 1992. Isoflavanones from the heartwood of *Swartzia polyphylla* and their antibacterial activity against cariogenic bacteria. Chem. Pharm. Bull. 40, 2970–2974.
Plaza, A., Montoro, P., Benavides, A., Pizza, C., Piacente, S., 2005. Phenylpropanoid glycosides from *Tynanthus panurensis*: characterization and LC-MS quantitative analysis. J. Agric. Food Chem. 53, 2853–2858.
Ruiz, L., Ruiz, L., Maco, M., Cobos, M., Roumy, V., 2011. Plants used by native Amazonian groups from the Nanay River (Peru) for the treatment of malaria. J. Ethnopharmacol. 133, 917–921.
Santos, S.A.O., Freire, C.S.R., Domingues, M.R.M., Silvestre, A.J.D., Pascoal Neto, C., 2011. Characterization of phenolic components in polar extracts of *Eucalyptus globulus* Labill. bark by high-performance liquid chromatography-mass spectrometry. J. Agric. Food Chem. 59, 9386–9393.
Schmeda-Hirschmann, G., Delporte, C., Valenzuela-Barra, G., Silva, X., Vargas, A., G., Lima, B., Feresin, G.E., 2014. Anti-inflammatory activity of animal oils from the Peruvian Amazon. J. Ethnopharmacol. 156, 9–15.
Sharma, M., Arya, D., Bhagour, K., Gupta, R.S., 2017. Natural aphrodisiac and fertility enhancement measures in males: a review. CMRP 7, 51–58.
Singh, S., Prasad, B., Savaliya, A.A., Shah, R.P., Gohil, V.M., Kaur, A., 2009. Strategies for characterizing sildenafil, vardenafil, tadalafil and their analogues in herbal dietary supplements, and detecting counterfeit products containing these drugs. TrAC 28, 13–28.
Sobeh, M., Mahmoud, M.F., Abdelfattah, M.A.O., Cheng, H., EI-Shazly, A.M., Wink, M., 2018. A proanthocyanidin-rich extract from *Cassia abbreviate* exhibits antioxidant and hepatoprotective activities in vivo. J. Ethnopharmacol. 213, 38–47.
Stalmach, A., Mullen, W., Pecorari, M., Serafini, M., Crozier, A., 2009. Bioavailability of C-linked dihydrochalcone and flavanone glucosides in humans following ingestion of unfermented and fermented rooibos teas. J. Agric. Food Chem. 57, 7104–7111.
Tang, X., Olatunji, O.J., Zhou, Y., Hou, X., 2017. In vitro and in vivo aphrodisiac properties of the seed extract from *Allium tuberosum* on corpus cavernosum smooth muscle relaxation and sexual behavior parameters in male Wistar rats. BMC Complement. Altern. Med. 17, 510.
van Andel, T., Mitchell, S., Volpato, G., Vandebroek, I., Swier, J., Ruysschaert, S., Jimenez, C.A.R., Raes, N., 2012. In search of the perfect aphrodisiac: parallel use of bitter tonics in West Africa and the Caribbean. J. Ethnopharmacol. 143, 840–850.
Vásquez-Ocmín, P., Cojean, S., Rengifo, E., Suyyagh-Albouz, S., Guerra, C.A.A., Pmel, S., Cabanillas, B., Mejía, K., Loiseau, P.M., Figadere, B., Maciuk, A., 2018. Antiprotozoal activity of medicinal plants used by Iquitos-Nauta road communities in Loreto (Peru). J. Ethnopharmacol. 210, 372–385.
West, E., Krychman, M., 2015. Natural aphrodisiacs - a review of selected sexual enhancers. Sex Med. Rev. 3, 279–288.
Zheng, B.L., He, K., Kim, C.H., Rogers, L., Shao, Y., Huang, Y.Z., Lu, Y., Yan, S.J., Qien, L.C., Zheng, Q.Y., 2000. Effect of a lipidic extract from *Lepidium meyenii* on sexual behavior in mice and rats. Urology 55, 598–602.
Zhu, M., Dong, X., Guo, M., 2015. Phenolic profiling of *Duchesnea indica* combining microporous resin chromatography (MRC) with HPLC-ESI-MS/MS and ESI-IT-MS. Molecules 20, 22463–22475.