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Non-volatile leaves compounds LC-MS profile of the two Cuban *Piper aduncum* L. subspecies

[Perfil de CL-SM de compuestos no volátiles de las hojas de las dos subspecies cubanas de *Piper aduncum* L.]

Orlando A. Abreu-Guirado[1], Kenn Foubert[2], Julio Escalona-Arranz[3,4], Daniel Méndez-Rodríguez[1], Andrés Rivera-Mondragón[5,6], Paul Cos[2], Ania Ochoa-Pacheco[3] & Luc Pieters[2]

[1]Facultad de Ciencias Aplicadas, Universidad de Camagüey Ignacio Agramonte Loynaz, Camagüey, Cuba
[2]Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Belgium
[3]Departmento de Farmacia, Facultad de Ciencias Naturales y Exactas, Universidad de Oriente, Santiago de Cuba, Cuba
[4]Departamento de Ciencias Farmacéuticas, Facultad de Ciencias. Universidad Católica del Norte, Antofagasta, Chile
[5]Departamento de Química Medicinal y Farmacognosia. Facultad de Farmacia, Universidad de Panamá, Panamá
[6]Instituto Especializado de Análisis, Universidad de Panamá, Panamá

Reviewed by:
Eduardo Dellacassa
Universidad de La Republica
Uruguay

Valdir Florencio da Veiga
Military Engineering Institute
Brazil

Correspondence:
Luc PIETERS
luc.pieters@uantwerpen.be

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Abstract: The medicinal plant *Piper aduncum* was segregated in Cuba as *P. aduncum* subsp. *ossanum* and *P. aduncum* subsp. *aduncum*, the latter has not been phytochemically investigated in Cuba. This study aims to compare profiles of non-volatile compounds of the aqueous and ethanolic extracts of Cuban *Piper aduncum* subspecies leaves. From both subspecies were prepared a decoction and, an ethanolic extract that yield two fractions. Analysis was performed by UPLC-ESI/QTOF/MS. Standard reference compounds were assessed by comparison with MS data of the plants extracts. Also, tentatively assigned compounds were proposed by matching m/z and fragmentation patterns with *P. aduncum* published compounds. Main compounds in decoction and aqueous ethanolic fraction of both subspecies were characterized as flavon-*C*-glycosides. Isoorientin was not previously reported in *P. aduncum*. Oleaginous ethanolic extracts differs among subspecies and peaks are coincident with benzoic acid derivate compounds. Results can serve for quality control of *P. aduncum* in Cuba.

Keywords: Cuban Medicinal Plants; Flavonoids; Flavon-C-glycosides; Subspecies; *Piper aduncum*

Resumen: La planta medicinal *Piper aduncum*, fue segregada en Cuba como *P. aduncum* subsp. *ossanum* y *P. aduncum* subsp. *aduncum*, la última carece de estudios fitoquímicos en Cuba. El objetivo fue comparar los perfiles de los compuestos no volátiles de extractos acuosos y etanólicos de hojas de las subespecies cubanas de *P. aduncum*. De ambas subespecies se prepararon decocciones y extractos etanólicos que rindieron dos fracciones, que fueron analizadas mediante UPLC-ESI/QTOF/MS. Los MS obtenidos se evaluaron respecto a compuestos de referencia y tentativamente se asignaron compuestos comparando con los reportados en *P. aduncum*. Los perfiles de LC-MS de decocciones y fracciones acuosas fueron similares en ambas subespecies, los compuestos mayoritarios fueron *C*-glucósidos de flavonas. La isorientina no se ha reportado antes en *P. aduncum*. Las fracciones oleaginosas difieren entre las subespecies, sus picos coinciden con derivados del ácido benzoico. Estos resultados pueden servir para el control de calidad de *P. aduncum* en Cuba.

Palabras clave: Flavonoides; C-glucósidos de flavonas; *Piper aduncum*; Plantas Medicinales Cubanas; Subespecies

INTRODUCTION

Piperaceae is a plant family that includes about two thousand species. *Piper* is one of the largest genera with more than 1000 species growing in tropical and subtropical regions (Saralegui, 2004). This genus is abundant in diverse metabolites like alkaloids, amides, benzoic acid derivatives, chromenes, lignans and neolignans, phenylpropanoids, and polyketides (Parmar *et al*., 1997; Kato & Furlan, 2007).

In Cuba, as part of the great biodiversity, 17 *Piper* species have been described, and most of them are endemic to the country. *Piper aduncum* L. (*P. angustifolium* R. et Pav., *P. elongatum* Vahl.), known in Cuba as 'Platanillo de Cuba', 'Guayuyo' and 'Canilla de muerto', is distributed in Mexico, Central America, South America and The Antilles (Saralegui, 2004).

The studies on the taxonomy of Piperaceae for *La Flora de la República de Cuba*, concluded that two infraspecific *taxa* of *P. aduncum* occur in Cuba with a particular geographical distribution: *P. aduncum* L. subsp. *aduncum* in the east region of the island, on the other hand, the endemic *P. aduncum* subsp. *ossanum* (C. CD.) Saralegui, that grows in the west and central region of the country up to Camagüey Province, including Isla de la Juventud (Figure No. 1) (Saralegui, 2004). This rank for these *taxa* is maintained in the current checklist of vascular plants of Cuba (Greuter & Rankin, 2022).

Hence, considering possible similarities and differences in phytochemical profiles, when investigated *P. aduncum* in Cuba it is important to consider the phytogeographical origin of the plant material (Abreu *et al*., 2012).

Figure No. 1
Infraspecific phytogeographical distribution of *Piper aduncum* in Cuba

P. aduncum in Cuba and other countries is traditionally recognized as having activity against skin and genito-urinary infections, gastrointestinal disorders, as a hemostatic, astringent, and diuretic, usually is administered orally as a decoction, or topically as a cataplasm (Roig, 1945; Morton, 1981; Seoane, 1984; Fuentes *et al*., 1985; Fuentes & Granda, 1988; Duke & Vázquez, 1994; Cruz, 1995). However, ethnobotanic investigations in Cuba only refers to *P. aduncum* subsp. *ossanum* (Seoane, 1984; Fuentes *et al*., 1985; Fuentes & Granda, 1988), with the exception of a use report for flu for *P. aduncum* subsp. *aduncum* (Riverón-Giró *et al*., 2015). That is why, the comparison of phytochemical profiles of both subspecies is also of ethnobotanical interest.

As non-volatile compounds, flavonoids, benzoic acid derivatives, chromenes, lignans and neolignans, phenylpropanoids and polyketides have been isolated from this specie (Sengupta & Ray, 1987; Parmar *et al*., 1997; Parmar *et al*., 1998). Essential oil has been widely investigated in *P. aduncum*, and at least nine essential oil chemotypes have been described for *P. aduncum* (Gutiérrez *et al*., 2016; Monzote *et al*., 2017). In benzoic acid derivates isolated from *P. aduncum* have been determined antibacterial, antifungal and antiparasitic activities, i.e. nervogenic acid, aduncumene, 3-geranyl-4-hydroxybenzoic, methyl-4-hydroxy-3-(3'-methyl-2'-butenyl)-benzoate, 3-(3'-7'-dimethyl- 2'-6'-octadienyl)-4-methoxy-benzoic acid, methyl 4-hydroxy-3-(3'-methyl-2'-butenyl)benzoate and 4-hydroxy-3-(3-methyl-2-butenoyl)-5-(3-methyl-2-

butenyl)-benzoic acid (Orjala *et al*., 1993; Okunade *et al*., 1997; Lago *et al*., 2004; Flores *et al*., 2009; Lago *et al*., 2009) and in the essential oil (Navickiene *et al*., 2006; Duarte *et al*., 2007; Lara *et al*., 2012; da Silva, 2021; Gutiérrez *et al*., 2016; Monzote *et al*., 2017). Until now, 2″-*O*-rhamnosyl-4′-*O*-methyl-vitexin, a flavone glycoside identified in *P. aduncum* subsp. *ossanum* (Larionova *et al*., 2010), is the only reference to *P. aduncum* non-volatile compounds in Cuba, which has shown anti-inflammatory and gastric antiulcerogenic effects (Apecechea *et al*., 2000; González *et al*., 2003; Martínez *et al*., 2004).

P. aduncum subsp. *aduncum* has not been phytochemically studied in Cuba, thus it is not possible to compare non-volatile compounds from this *taxon* with *P. aduncum* subsp. *ossanum*. This study aims to compare the profiles of the non-volatile constituents of aqueous and ethanolic extracts of Cuban *Piper aduncum* subspecies by UPLC-MS.

MATERIAL AND METHODS

Plant material and extracts

Leaves of *P. aduncum* subsp. *ossanum* (C.CD.) Saralegui and *P. aduncum* L. subsp. *aduncum* were collected in La Cantera locality at Sierra de Cubitas Municipality (Camagüey Province), and in the El Caney de las Mercedes village at Bartolomé Masó Municipality (Granma Province), (Lat 21.60629, Lon -77.83807 and Lat 20.15662, Lon -76.94703 respectively). In the second locality pluviosity is higher. Collection of both *taxa* took place before 8:00 a.m. in December of 2018. The plant material was authenticated by I. Méndez, curator of the herbarium of the University of Camagüey, where the specimens were deposited (voucher numbers: PNP-0006 (HIPC) and PNP-0007 (HIPC), respectively). The plant material was shade air-dried and subsequently triturated.

Water extracts were prepared by decoction. Ten (10) g of the plant material was boiled in 200 mL of water for 15 minutes. Ethanolic extracts were prepared by maceration of crude drug with ethanol: water (70:30) for 10 days. Decoctions and ethanolic extracts of both subspecies were concentrated at 40°C in a rotary evaporator, freeze-dried and kept at 4°C until use.

Preparation of solutions

Solutions of plant extracts and reference compounds were prepared before injection at a concentration of 25 and 5 µg mL^{-1} respectively, in LC/MS-grade methanol (80%). Reference compounds were mainly flavonoids, organic acids and benzoic acid derivatives, and included 4-acetyl-benzoic acid, apigenin, benzoic acid, caffeic acid, +/- catechin, chlorogenic acid, 3,4-dihydroxy-benzoic acid, emodin, epicatechin, ferulic acid, 4-hydroxy-benzoic acid, isoquercetin, isorhamnetin, isoorientin, isovitexin, kaempferol, luteolin, naringenin, orientin, *o*-coumaric acid, *p*-coumaric acid, procyanidin B, protocatechuic acid, quercetin, quercitrin, rutin, salicin, taxifolin, *trans*-cinnamic acid, vanillic acid, vanillin, vitexin and vitexin 2-*O*-rhamnoside. All of them were purchased from Sigma Aldrich®.

UPLC-QTOF-MS/MS conditions

For LC-MS analysis and accurate mass measurements, a Xevo G2-XS QTOF spectrometer (Waters, Milford, MA, USA) coupled with an ACQUITY LC system was used. For analysis, 5 µL of each solution were injected into an ACQUITY UPLC BEH Shield RP18 column (100 mm × 2.1 mm, 1.7 µm; Waters, Milford, MA, USA). The mobile phase solvents consisted of nano-pure H_2O acidified with 0.1% formic acid (A) and ACN + 0.1% formic acid (B). The flow rate was set at 0.4 mL min-1, and the gradient was set as follows (min/B%): 0.0/2.0, 1.0/2.0, 14.0/26.0, 24.0/65.0, 26/100.

Spectra were recorded in negative electrospray ionization (ESI) mode with spectra acquired over a mass-to-charge ratio (*m/z*) range from 50-1500, using nitrogen as a drying and nebulizing gas and resolving power was set at 22,000 FWHM. Leucine-encephalin was used as lock mass. The spray voltage was set at -0.8 kV; cone gas flow and desolvation gas flow at 50.0 L h^{-1} and 1000.0 L h^{-1}, respectively; source temperature and desolvation temperature were set at 120°C and 550°C, respectively. The peaks and spectra were interpreted using the Masslynx 4.1® software. A single analysis was performed for obtaining both the precursor and fragment ions.

The presence of particular targeted compounds (flavone-*C*-glycosides) was confirmed by matching retention time, *m/z* values and MS^E fragmentation pattern with standard reference compounds. Compounds were also tentatively identified by comparison of the obtained *m/z* values and MS^E fragmentation patterns with published data of *P. aduncum* non-volatile compounds. In the case

of flavonoids, the position of glycosides was assigned according to the more probable C-C or O-C bond position in flavones as reviewed by Vukics and Guttman (2010).

Levels of confidence were assigned according to Schymanski *et al*. (2014): Level 1 (L1) - Structure confirmed by a reference compound; level 2a (L2a) - Probable structure by literature or library spectrum match; level 3 (L3) - tentative identification based on MS, MS^2 experimental data.

RESULTS AND DISCUSSION

In the process of vacuum concentration ethanolic extracts of both subspecies in the rotary evaporator, when concentrated to 1/3 of initial volume, it split in two phases, a brown aqueous fraction (AF) with a sweet smell and syrupy texture, and, a greenish spice-smelling oily fraction (OF) attached to the flask glass surface, thus easily separated by decantation.

The total ion chromatograms (TICs) of *P. aduncum* subsp. *ossanum* and *P. aduncum* subsp. *aduncum* decoction and AF are depicted in Figures No. S1-S2 (Supplementary Information No. 1), TICs of the matching reference standards are shown in Figure No. S3, while the OF TIC is displayed in Figure No. S4. The non-volatile chemical profiles of the decoction and AF in both *P. aduncum* subspecies provided a very similar chromatographic fingerprint. In contrast, differences between both subspecies chemical pattern in OF samples were found.

Data on retention time, molecular formula, *m/z*, MS1 and MS^E signals of analyzed extracts are shown in Tables No. S1-S3 (Supplementary Information No. 2). Also, in Tables No. S1-S3 compounds are semi-quantitatively annotated according to their relative abundance. In general, were observed a higher relative abundance of ions in *P. aduncum* subs. *ossanum* than those found in *P. aduncum* subsp. *aduncum*, this could be related to environmental factors. Further quantitative assessment of the main metabolites in both Cuban subspecies are required.

Main peaks in both *P. aduncum* subspecies decoction and AF correspond to sixteen *C*-glycosyl substituted flavones listed in Table No 1, including apigenin (**6**, **9**, **11**) luteolin (**1**, **2**, **4**, **5**) and its 4′ or 7-methoxyflavone derivates (**3**, **7**, **8**, **10**, **12-16**) (Figures No. S1-S4). These substituted flavones show mass losses in MS^E spectra characteristic for *C*-glycosides with hexose and hexose-deoxyhexose ($-\ ^{0,1}X$, $-\ ^{0,2}X$, $-\ ^{0,3}X$, $-\ ^{0,2}X - H_2O$) and also for *O*-glycosides ($-$ Yi) (Vukics & Guttman, 2010). For example, 8-(*C*-glucosyl)isorientin (**1**), 2″-(*O*-rhamnosyl)orientin/isoorientin (**3**) revealed $[M - H]^-$ ions at m/z 609.1456 and 593.1505, respectively. MS^E product ion spectra of **1** and **3** showed characteristic losses of 90 ($-\ ^{0,3}X$), 120 ($-\ ^{0,2}X$) and 150 ($-\ ^{0,1}X$) which suggested the cleavage of a *C*-glucosyl (hexose) bond. Furthermore, **3** gave a product ion at m/z 447.09, corresponding to a neutral loss of an *O*-rhamnoside moiety $[M - H - 146\ (-Yi)]^-$.

The observed MSE patterns allowed us to confirm the presence of aglycones and to establish glycosides as mono-*C*-glycosides or di-*C*-glycosides, by matching patterns of flavonoid aglycones (Ag) with their glycosides; namely [Ag + 41]- and [Ag + 71]- for mono-*C*-glycosides and [Ag + 83]- and [Ag + 113]- for di-*C*-glycosides, as Vukics and Guttman (2010) suggested (Tables No. S1-S2 in Supplementary Information No. 2).

In the decoction and AF extracts of both *P. aduncum* subspecies five flavon-*C*-glycosides were unambiguously identified (L1) by comparison with authentic reference standards of 4-6, 9, 11. Our study demonstrated the presence of isoorientin (5) in *P. aduncum* by the first time, and for the best of our knowledge in the genus *Piper*. The other tentative flavon-*C*-glycosides were assigned by matching MS data and MSE fragmentation patterns with published information on *P. aduncum* constituents (L2a). Isospinosin (13) was found only in AF fraction. Compounds 4, 6, 9-16 have previously been reported in *P. aduncum* (Masuoka *et al*., 2003; Larionova *et al*., 2010; Thao *et al*., 2016). The other tentatively identified compounds were not reported before in *P. aduncum* (L3). Structures of proposed flavon-C-glycosides are depicted in Figure No. 2.

The TIC of the decoction and AF of both *P. aduncum* subspecies shows that the main peaks correspond to **14**, **4**, **5**, **6**, **9** and **11**. Other tentatively assigned main compounds from these extracts are the positional isomers isoswertiajaponin and 4′-*O*-methylorientin (**10**, **12**), cytisoside and swertisin (**15**, **16**), previously isolated in *P. aduncum* (Masuoka *et al*., 2003; Thao *et al*., 2016).

Table No. 1.
Flavon-*C*-glycosides in the decoctions and aqueous fraction of ethanolic extracts of Cuban *Piper aduncum* subsp. *ossanum* and *Piper aduncum* subsp. *aduncum*

Compound identification	Molecular Formula	Rt	Accurate Mass (*m/z*) in negative mode	CL
(**1**) 8-(*C*-β-glucosyl)isorientin	$C_{27}H_{30}O_{16}$	7.04	609.1446	L3
(**2**) Vicenin-2	$C_{27}H_{30}O_{15}$	7.70	593.1510	L3
(**3**) 2"-(*O*-rhamnosyl)orientin/isoorientin	$C_{21}H_{20}O_{11}$	8.93	593.1505	L3
(**4**) Orientin*	$C_{27}H_{30}O_{15}$	8.93	447.0933	L1
(**5**) Isoorientin	$C_{21}H_{20}O_{11}$	9.10	447.0933	L1
(**6**) 2"-(*O*-rhamnosyl)vitexin*	$C_{27}H_{30}O_{14}$	9.52	577.1559	L1
(**7**) 8-(*C*-glucosyl)-4′-*O*-metylisovitexin	$C_{28}H_{32}O_{15}$	9.52	607.1666	L3
(**8**) 6-(*C*-glucosyl)-7-*O*-methylvitexin	$C_{28}H_{32}O_{15}$	9.52	607.1666	L3
(**9**) Vitexin*	$C_{21}H_{20}O_{10}$	9.62	431.0981	L1
(**10**) Isoswertiajaponin*/4′-*O*-methylorientin*	$C_{22}H_{22}O_{11}$	10.23	461.1084	L2a
(**11**) Isovitexin*	$C_{21}H_{20}O_{10}$	10.50	431.0981	L1
(**12**) Isoswertiajaponin*/4′-*O*-methylorientin*	$C_{22}H_{22}O_{11}$	10.67	461.1084	L2a
(**13**) Isospinosin*&	$C_{28}H_{32}O_{15}$	10.97	607.1647	L2a
(**14**) 2″-(*O*-rhamnosyl)-4′-*O*-methylvitexin*	$C_{28}H_{32}O_{14}$	11.29	591.1718	L2a
(**15**) Cytisoside*/Swertisin*	$C_{22}H_{22}O_{10}$	11.72	445.1142	L2a
(**16**) Cytisoside*/Swertisin*	$C_{22}H_{22}O_{10}$	13.18	445.1142	L2a

***: Previously reported compound in *P. aduncum*; &: only found in AF ethanolic extracts; CL: Confidence level**

C-flavones	R_1	R_2	R_3	R_4	R_5
(**9**) Vitexin	H	H	Gluc	H	H
(**6**) 2"-(*O*-rhamnosyl)vitexin	H	H	Rha-Glc*	H	H
(**14**) 2″-(*O*-rhamnosyl)-4′-*O*-methylvitexin	H	H	Rha-Glc*	CH_3	H
(**11**) Isovitexin	Glc	H	H	H	H
(**2**) Vicenin-2	Glc	H	Glc	H	H
(**15**) Cytisoside	H	H	Glc	CH_3	H
(**16**) Swertisin	Glc	CH_3	H	H	H
(**8**) 6-(*C*-glucosyl)-7-*O*-methylvitexin	Glc	CH_3	Glc	H	H
(**13**) Isospinosin&	H	CH_3	Glc- Glc*	H	H
(**7**) 8-(*C*-glucosyl)-4′-*O*-metylisovitexin	Glc	H	Glc	CH_3	H
(**4**) Orientin	H	H	Glc	H	OH
(**10**) Isoswertiajaponin	H	CH_3	Glc	H	OH
(**12**) 4′-O-methylorientin	H	H	Glc	CH_3	OH
(**5**) Isoorientin	Glc	H	H	H	OH
(**1**) 8-(*C*-glucosyl)isorientin	Glc	H	Glc	H	OH
(**3**) 2"-(*O*-rhamnosyl)orientin/isoorientin	Rha-Glc*/H	H	Rha-Glc*/H	H	OH

&: only found in AF ethanolic extracts; Glc: glucose; Rha: rhamnose; *: (1 → 2).

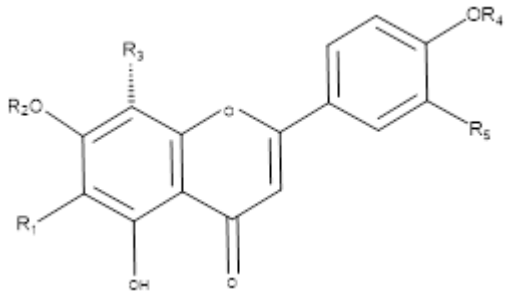

Figure No. 2
Chemical structure of assigned flavone *C*-glycosides in both Cuban *Piper aduncum* subspecies

It is the first time that the tentatively identified flavones **1-3**, **7**, **8** are reported in *P. aduncum*. Further research confirming the identity of all tentatively identified compounds should be carried out.

Several biological activities have been described for the flavones contained in the analyzed extracts. Antioxidant, antimicrobial, anti-inflammatory, antinociceptive, cardioprotective and neuroprotective effects of vitexin, isovitexin, orientin and isorientin have been reviewed by He *et al*. (2016), and Lam *et al*. (2016). Vicenin-2, 2″-(*O*-rhamnosyl)orientin/isoorientin, cytisoside and swertisin were also reported to exhibit diverse biological effects (Wang *et al*., 2012; Xiao *et al*., 2016).

The unusual glycoside 2″-*O*-rhamnosyl-4′-*O*-methylvitexin (**14**) (L2a), assigned by ^{1}H and ^{13}C NMR, was the main metabolite found by Larionova *et al*. (2010), in *P. aduncum* subsp. *ossanum* collected in Havana, Cuba. This flavone was found conspicuously in the decoction and AF of *P. aduncum* subsp. *ossanum* and *P. aduncum* subsp. *aduncum*. Thao *et al*. (2016), also isolated this flavone and other apigenin and luteolin *C*-glycosides in *P. aduncum* from West Java, Indonesia, suggesting flavon-*C*-glycosides as markers for this plant. Particularly, isoorientin could be also a new compound of chemophenetic significance in *P. aduncum*.

Among more than 100 *P. aduncum* isolated non-volatile compounds compiled in our database, 2″-*O*-rhamnosyl-4′-*O*-methylvitexin, is the only compound isolated starting from a water extract (Larionova et al., 2010), and this is consistent with the presence of this flavone among the main peaks in the decoction and AF in both Cuban *P. aduncum* subspecies. 2″-*O*-rhamnosyl-4′-*O*-methylvitexin isolated from *P. aduncum* subsp. *ossanum* was reported with anti-inflammatory and antiulcerogenic activity (Apecechea *et al*., 2000; González *et al*., 2003; Martínez *et al*., 2004).

Therefore, the pool of *C*-flavone glycosides, as the main compounds in the extracts obtained by a the decoction from both Cuban *P. aduncum* subspecies, can be associated with the profuse number of records as a medicinal plant in Cuba, since this is the main traditional form of preparation of *P. aduncum* leaves for oral administration and also in some case for topical use (Roig, 1945; Morton, 1981; Seoane, 1984; Fuentes *et al*., 1985; Fuentes & Granda, 1988; Duke & Vázquez, 1994; Cruz, 1995). Likewise, in animal models in Peru, gastroprotective, antisecretory and hepatoprotective effects of ethanolic extract of leaves from *P. aduncum* and fractions were demonstrated (Arroyo *et al*., 2012; Arroyo *et al*., 2013).

Main peaks in both *P. aduncum* subspecies OF extracts (Table No. S3 in Supplementary Information 2), correspond to tentatively identified compounds derived from benzoic acid (Table No. 2), although remarkably, the flavanone pinocembrin (**18**) (5,7-dihydroxyflavanone) is among the main compounds in *P. aduncum* subsp. *ossanum*. In Figure No. 3 the structure of the suggested compounds are shown.

Table No. 2
Compounds (benzoic acid derivatives and pinocembrin) in oleaginous fraction of ethanolic extracts of Cuban *Piper aduncum* subsp. *ossanum* and *Piper aduncum* subsp. *aduncum*

Compound identification	Molecular Formula	Rt	Accurate Mass (*m/z*) in negative mode	CL
17.*4-hydroxy-3-(3-methyl-2-butenoyl)-5-(3-methyl-2-butenyl)-benzoic acid	$C_{17}H_{20}O_4$	18.83	287.1281	L2a
18.*Pinocembrin&	$C_{15}H_{12}O_4$	19.06	255.0656	L2a
19.*Methyl-4-hydroxy-3-(3'-methyl-2'-butenyl)-benzoate	$C_{13}H_{16}O_3$	20.02	219.1017	L2a
20.*Nervogenic acid/*3-geranyl-4-hydroxybenzoic acid (geranyl-hydroxybenzoate)	$C_{17}H_{22}O_3$	21.54	273.1490	L2a
21.*Nervogenic acid/*3-geranyl-4-hydroxybenzoic acid (geranyl-hydroxybenzoate)	$C_{17}H_{22}O_3$	21.71	273.1490	L2a

***: Previously reported compound in *P. aduncum*; &: This compound is a flavanone; CL: Confidence level**

In OF extracts, all suggested compounds were previously isolated in *P. aduncum*. Pinocembrin was previously isolated from inflorescences and leaves of *P. aduncum* in Brazil (Lago *et al*., 2009; De Castro *et al*., 2015), this flavanone also was found in the leaves of other neotropical *Piper* species, i.e. *P. gaudichaudianum*, *P. hostmannianum*, *P. lanceaefolium* and *P. sarmentosum* (López *et al*., 2002; Lago *et al*., 2004; Ruddock *et al*., 2011). Besides, antimicrobial, anti-inflammatory, antioxidant, and anticancer activity has been reported in pinocembrin (Tundis *et al*., 2018; Shen *et al*., 2019).

In OF dissimilarity between both subspecies is pronounced. Some unknown or probable compounds (L2a, L3) that were present in *Piper aduncum* subsp. *ossanum* OF, were not detected in *Piper aduncum* subsp. *aduncum*, and vice versa (Table No. S4 in Supplementary Information 2). These differences can be due to abiotic causes like soil or weather, or by genetic factors. Additional investigations are needed to explain differences between both *taxa* profiles. The most abundant peaks in *P. aduncum* subsp. *aduncum* OF are compounds **17** and **19**. The peaks at 21.54 and 21.71 min with the same m/z, that were noted in both subspecies, are proposed as isomers compounds **20** and **21** (Table No. S3 in Supplementary Information 2).

All these benzoic acid derivates previously isolated in *P. aduncum* have been reported to exhibit antibacterial, antifungal and antiparasitic activity (Orjala *et al*., 1993; Okunade *et al*., 1997; Flores et al., 2009). Therefore, these compounds also could be related to ethnomedicinal uses in Cuba of *P. aduncum* as antimicrobial (Roig, 1945; Morton, 1981; Seoane, 1984; Fuentes *et al*., 1985; Fuentes & Granda, 1988).

CONCLUSIONS

Chemical profiles of the non-volatile compounds in *P. aduncum* subsp. *ossanum* and *P. aduncum* subsp. *aduncum* indicate the presence of known and novel flavone-*C*-glycosides, as well as benzoic acid derivatives and a flavanone. Isoorientin was identified for the first time in *P. aduncum* and in the genus *Piper*. This is the first attempt to determine the phytochemical profile of Cuban *P. aduncum* subspecies. The similarities and differences between the phytochemical patterns of non-volatile compounds in extracts of Cuban subspecies of *P. aduncum* can be used as fingerprint for quality control of herbal medicines developed from both subspecies, and can be associated to traditional medicinal uses and confirmed pharmacological activities of these *taxa*.

Supplementary information

The online version contains supplementary material available at https://doi. org/

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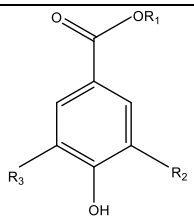
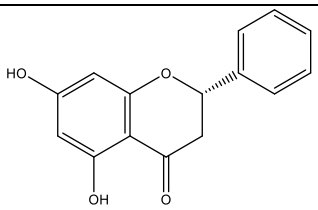
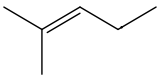
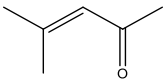
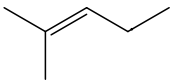
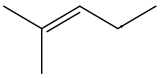
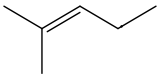
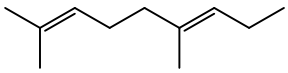
Benzoic acid derivatives	R_1	R_2	R_3
(18) Pinocembrin			
(19) Methyl-4-hydroxy-3-(3'-methyl-2'-butenyl)-benzoate			
(17) 4-hydroxy-3-(3-methyl-2-butenoyl)-5-(3-methyl-2-butenyl)-benzoic acid	H		
(20) Nervogenic acid	H		
(21) 3-geranyl-4-hydroxybenzoic acid	H	H	

Figure No. 3
Chemical structure of assigned benzoic acid derivatives and pinocembrin in both Cuban *Piper aduncum* subspecies

REFERENCES

Abreu OA, Rodríguez AA, Morgado M, Cao LB. 2012. Farmacognosia, farmacobotánica, farmacogeografía y farmacoetimología del platanillo de Cuba (*Piper aduncum* subespecie *ossanum*). **Rev Cub Plant Med** 17: 181 - 193.

Apecechea MR, Larionova M, Salazar S, Abín G. 2000. Evaluación de la actividad antiulcerosa del 2"-*O*-ramnosil 4′-*O*-metil-vitexina de las hojas de *Piper ossanum*. **Rev Cub Med Milit** 29.

Arroyo J, Almora Y, Quino M, Raez E, Martínez J, Buendía J, Baca D, Hañari R. 2012. Efecto protector en cirrosis hepática inducida en ratas del extracto etanólico de las hojas de *Piper aduncum* comparado con silimarina. **An Fac med** 73: 85 - 91.

Arroyo J, Bonilla P, Moreno-Exebio L, Ronceros G, Tomás G, Huamán J, Raez E, Quino M, Rodríguez-Calzado J. 2013. Efecto gastroprotector y antisecretor de un fitofármaco de hojas de matico (*Piper aduncum*). **Rev Peru Med Exp Salud Pub** 30: 608 - 615.

Cruz JL. 1995. **Dicionario das plantas úteis do Brasil.** Bertrand, Rio de Janeiro, Brasil.

da Silva ACA, Matias EFF, Rocha JE, de Araújo ACJ, de Freitas TS, Campina FF, Costa MS, Silva LE, do Amaral W, Maia BHLNS, Ferriani AP, Bezerra CF, Iriti M, Coutinho HDM. 2021. Gas chromatography coupled to mass spectrometry (GC-MS) characterization and evaluation of antibacterial bioactivities of the essential oils from *Piper arboreum* Aubl., *Piper aduncum* L. e *Piper gaudichaudianum* Kunth. **Zeitschrift für Naturforschung C** 76: 35 - 42. **https://doi.org/10.1515/znc-2020-0045**

De Castro CCB, Costa OS, Laktin GT, de Carvalho PHD, Geraldo RB, de Moraes J, Pinto PLS, Couri MRC, Pinto PF, Da Silva Filho AA. 2015. Cardamonin, a schistosomicidal chalcone from *Piper aduncum* L. (Piperaceae) that inhibits *Schistosoma mansoni* ATP diphosphohydrolase. **Phytomedicine** 22: 921 - 928. **https://doi.org/10.1016/j.phymed.2015.06.009**

Duarte MCT, Leme EE, Delarmelina C, Soares AA, Figueira. GM, Sartoratto A. 2007. Activity of essential oils from Brazilian medicinal plants on *Escherichia coli*. **J Ethnopharmacol** 111: 197 - 201. **https://doi.org/10.1016/j.jep.2006.11.034**

Duke JA, Vázquez R. 1994. **Amazonian ethnobotanical dictionary**. CRS Press Inc. Boca Ratón, FL, USA.

Flores N, Jiménez IA, Giménez A, Ruiz G, Gutiérrez D, Bourdy G, Bazzocchi IL. 2009. Antiparasitic activity of prenylated benzoic acid derivatives from *Piper* species. **Phytochemistry** 70: 621 - 627. **https://doi.org/10.1016/j.phytochem.2009.03.010**

Fuentes VR, Granda MM. 1988. Estudios de la Medicina Tradicional en Cuba. III. **Rev Cub Farm** 22: 77 - 90.

Fuentes VR, Rodríguez M, Poucheaux M, Cabrera L, Lara S. 1985. Estudios en la medicina tradicional en Cuba. II. **Bol Res Plant Med** 5: 13 - 40.

González M, Larionova M, Martínez AM, Marrero O. 2003. Evaluación de la acción antiinflamatoria del producto FL-1. **Rev Cub Med Mil** 8.

Greuter W., Rankin R. 2022. **Vascular Plants of Cuba A Checklist.** Botanischer Garten und Botanisches Museum Berlin. Berlin. **https://doi.org/10.3372/cubalist.2022.1**

Gutiérrez Y, Montes R, Scull R, Sánchez A, Cos P, Monzote L, Setzer WN. 2016. Chemodiversity associated with cytotoxicity and antimicrobial activity of *Piper aduncum* var. *ossanum*. **Chem Biodivers** 13: 1715 - 1719. **https://doi.org/10.1002/cbdv.201600133**

He M, Min JW, Kong WL, He XH, Li JX, Peng BW. 2016. A review on the pharmacological effects of vitexin and isovitexin. **Fitoterapia** 115: 74 - 85. **https://doi.org/10.1016/j.fitote.2016.09.011**

Lara CR, Lopes de Oliveira G, Mota BCF, Fernandes MFG, Figueiredo LS, Martins ER, Moreira DL, Kaplan MAC. 2012. Antimicrobial activity of essential oil of *Piper aduncum* L. (Piperaceae). **J Med Plant Res** 6: 3800 - 3805. **https://doi.org/10.5897/JMPR12.738**

Kato MJ, Furlan M. 2007. Chemistry and evolution of the *Piperaceae*. **Pure Appl Chem** 79: 529 - 538. **https://doi.org/10.1351/pac200779040529**

Lago JH, Ramos CS, Casanova DCC, de Morandim A, Bergamo DCB, Cavalheiro AJ, Bolzani VDS, Furlan M, Guimarães EF, Young MCM, Kato MJ. 2004. Benzoic acid derivatives from *Piper* species and their fungitoxic activity against *Cladosporium cladosporioides* and *C. sphaerospermum*. **J Nat Prod** 67: 1783 - 1788. **https://doi.org/10.1021/np030530j**

Lago JH, Chen A, Young MCM, Guimaraes EF, de Oliveira A, Kato MJ. 2009. Prenylated benzoic acid derivatives from *Piper aduncum* L. and *P. hostmannianum* C. DC. (Piperaceae). **Phytochem Lett** 2: 96 - 98. **https://doi.org/10.1016/j.phytol.2009.01.001**

Lam KY, Pick A, Ling L, Koh RY, Wong YP, Say YH. 2016. A review on medicinal properties of orientin. **Adv Pharmacol Sci https://doi.org/10.1155/2016/4104595**

Larionova M, Spengler I, Nogueira C, Quijano L, Ramírez-Gualito K, Cortés-Guzmán F, Cuevas G, Calderón JS. 2010. A *C*-glycosylflavone from *Piper ossanum*, a compound conformationally controlled by CH/p and other weak intramolecular interactions. **J Nat Prod** 73: 1623 - 1627. **https://doi.org/10.1021/np100004v**

López A, Dong SM, Towers GHN. 2002. Antifungal activity of benzoic acid derivatives from *Piper lanceaefolium*. **J Nat Prod** 65: 62 - 64. **https://doi.org/10.1021/np010410g**

Martínez C, García M, Martínez SM, Larionova M, Sebazco C, Machín M, Ruiz V. 2004. Evaluación antiinflamatoria del flavonoide 2"-*O*-ramnosil 4′-*O*-metilvitexina en ratas. **Rev Cub Plant Med** 9

Masuoka C, Ono M, Ito Y, Nohara T. 2003. Antioxidative, antihyaluronidase and antityrosinase activities of some constituents from the aerial part of *Piper elongatum* Vahl. **Food Sci Technol Res** 9: 197 - 201. **https://doi.org/10.3136/fstr.9.197**

Monzote L, Scull R, Cos P, Setzer WN. 2017. Essential oil from *Piper aduncum*: Chemical analysis, antimicrobial assessment, and literature review. **Medicines** 4. **https://doi.org/10.3390/medicines4030049**

Morton J. 1981. **Atlas of medicinal plants of middle America**. Springfield, USA.

Navickiene HD, de Morandim AA, Alécio AC, Regasini LO, Bergamom DCB, Telascrea M, Cavalheiro AJ, Lopes MN, Bolzani VS, Furlan M, Marques MOM, Young MCM, Kato MJ. 2006. Composition and antifungal activity of essential oils from *Piper aduncum*, *Piper arboreum* and *Piper tuberculatum*. **Quim Nova** 29: 467 - 470. **https://doi.org/10.1590/S0100-40422006000300012**

Okunade AL, Hufford CD, Clark AM, Lentz D. 1997. Antimicrobial properties of the constituents of *Piper aduncum*. **Phytother Res** 11: 142 - 144.

Orjala J, Erdelmeier CAJ, Wright AD, Rali T, Sticher O. 1993. Five new prenylated p-hydroxy benzoic acid derivatives with antimicrobial and molluscicidal activity from *Piper aduncum* leaves. **Planta Med** 59: 546 - 551.

Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Tyagi JA, Prasad OD, Wengel AK, Olsen JCE, Boll PM. 1997. Phytochemistry of the genus *Piper*. **Phytochemistry** 46: 597 - 673. **https://doi.org/10.1016/S0031-9422(97)00328-2**

Parmar VS, Jain SC, Gupta S, Talwar S, Rajwanshi VK, Kumar R, Azim A, Malhotra S, Kumar R, Jain R, Sharma NK, Tyagi OD, Lawrie SJ, Errington W, Howarth OW, Olsen CE, Wengel J. 1998. Polyphenols and alkaloids from *Piper* species. **Phytochemistry** 49: 1069 - 1079. **https://doi.org/10.1016/S0031-9422(98)00208-8**

Riverón-Giró FB, Hernández-Montero Y, García-González A, Escalona-Domenech RY. 2015. La colección de plantas medicinales del Jardín Botánico de Holguín, Cuba: su importancia social y científica. **Rev Jardin Bot Nac** 36: 219 - 222.

Roig JT. 1945. **Plantas medicinales, aromáticas o venenosas de Cuba.** Ministerio de Agricultura, La Habana, Cuba.

Ruddock PS, Charland M, Ramirez S, López A, Towers GHN, Arnason JT, Liao M, Dillon J-AR. 2011. Antimicrobial activity of flavonoids from *Piper lanceaefolium* and other Colombian medicinal plants against antibiotic susceptible and resistant strains of *Neisseria gonorrhoeae*. **Sex Transm Dis** 38: 82 - 88. **https://doi.org/10.1097/OLQ.0b013e3181f0bdbd**

Saralegui H. 2004. **Piperaceae**. In: Greuter W, Rankin R. Ed. **Flora de la República de Cuba**, Serie A, Plantas Vasculares, Fascículo 9: 3 Königstein, Germany.

Schymanski EL, Jeon J, Gulde R, Fenner K, Ruff M, Singer HP, Hollender J. 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. **Environ Sci Technol** 48: 2097 - 2098. **https://doi.org/10.1021/es5002105**

Sengupta S, Ray AB. 1987. The chemistry of *Piper* species: a review. **Fitoterapia** 58: 147 - 166.

Seoane J. 1984. **El folclor médico de Cuba.** Editorial Ciencias Sociales, La Habana, Cuba.

Shen X, Liu Y, Luo X, Yang Z. 2019. Advances in biosynthesis, pharmacology, and pharmacokinetics of pinocembrin, a promising natural small-molecule drug. **Molecules** 24: 2323. **https://doi:10.3390/molecules24122323**

Thao NP, Luyen BTT, Widowati W, Fauziah N, Maesaroh M, Herlina T, Mansoor Z, Ali I, Koh YS, Kim YH. 2016. Anti-inflammatory flavonoid *C*-glycosides from *Piper aduncum* leaves. **Planta Med** 82: 1475 - 1481. **https://doi.org/10.1055/s-0042-108737**

Tundis R, Frattaruolo L, Carullo G, Armentano B, Badolato M, Loizzo MR, Aiello F, Cappello AR. 2018. An ancient remedial repurposing: synthesis of new pinocembrin fatty acid acyl derivatives as potential antimicrobial/anti-inflammatory agents. **Nat Prod Res** 33. **https://doi.org/10.1080/14786419.2018.1440224**

Vukics V, Guttman A. 2010. Structural characterization of flavonoid glycosides by multi-stage mass spectrometry. **Mass Spectrom Rev** 29: 1 - 16. **https://doi.org/10.1002/mas.20212**

Wang MJ, Yue YD, Tang F, Sun J. 2012. TLC screening for antioxidant activity of extracts from fifteen bamboo species and identification of antioxidant flavone glycosides from leaves of *Bambusa textilis*. **Molecules** 17: 12297 - 12311. **https://doi.org/10.3390/molecules171012297**

Xiao J, Capanoglu E, Jassbi, AR, Miron A. 2016. Advance on the flavonoid *C*-glycosides and health benefits. **Crit Rev Food Sci** 56: S29 - S45. **https://doi.org/10.1080/10408398.2015.1067595**

Supplementary Material No. 1
Total ion chromatogram of *Piper aduncum* subsp. ossanum and *P. aduncum* subsp. *aduncum* extracts and identified compounds

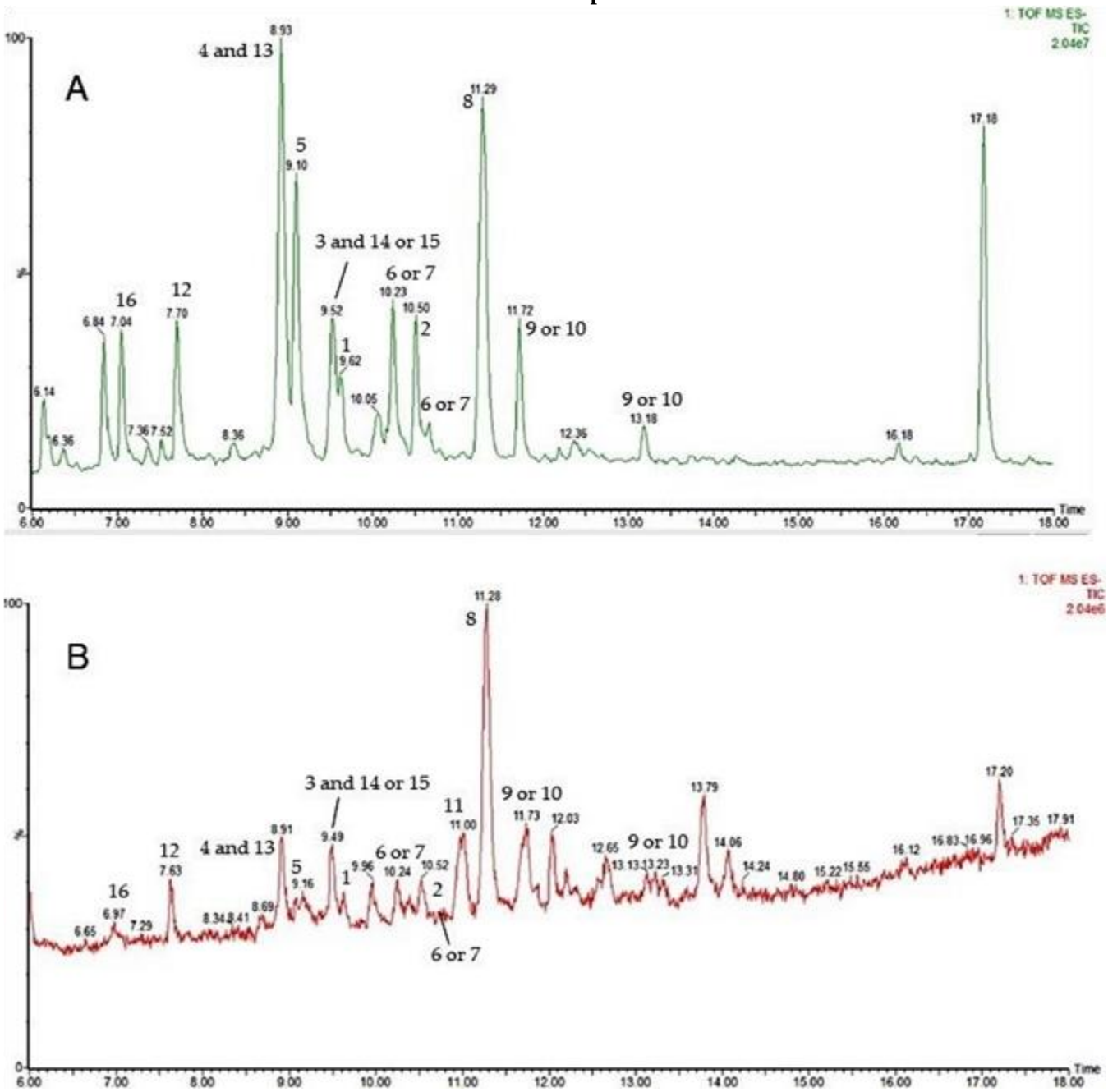


Figure S1
Total ion chromatogram of the decoction of Cuban *Piper aduncum* subsp. *ossanum* (A) and *Piper aduncum* subsp. *aduncum* (B)

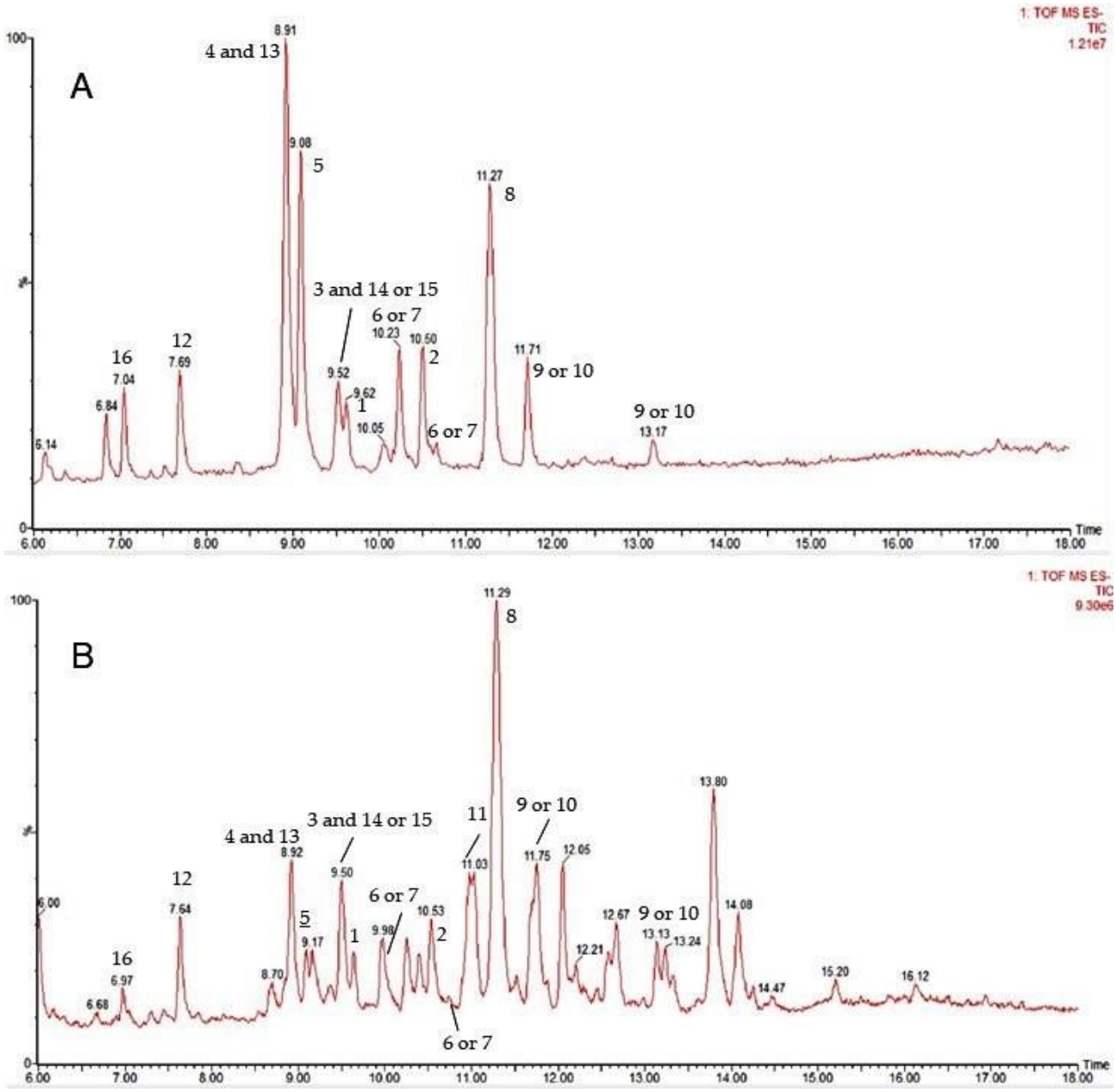


Figure S2
Total ion chromatogram of the ethanolic extract aqueous fraction (AF) of Cuban *Piper aduncum* subsp. *ossanum* (A) and *Piper aduncum* subsp. *aduncum* (B)

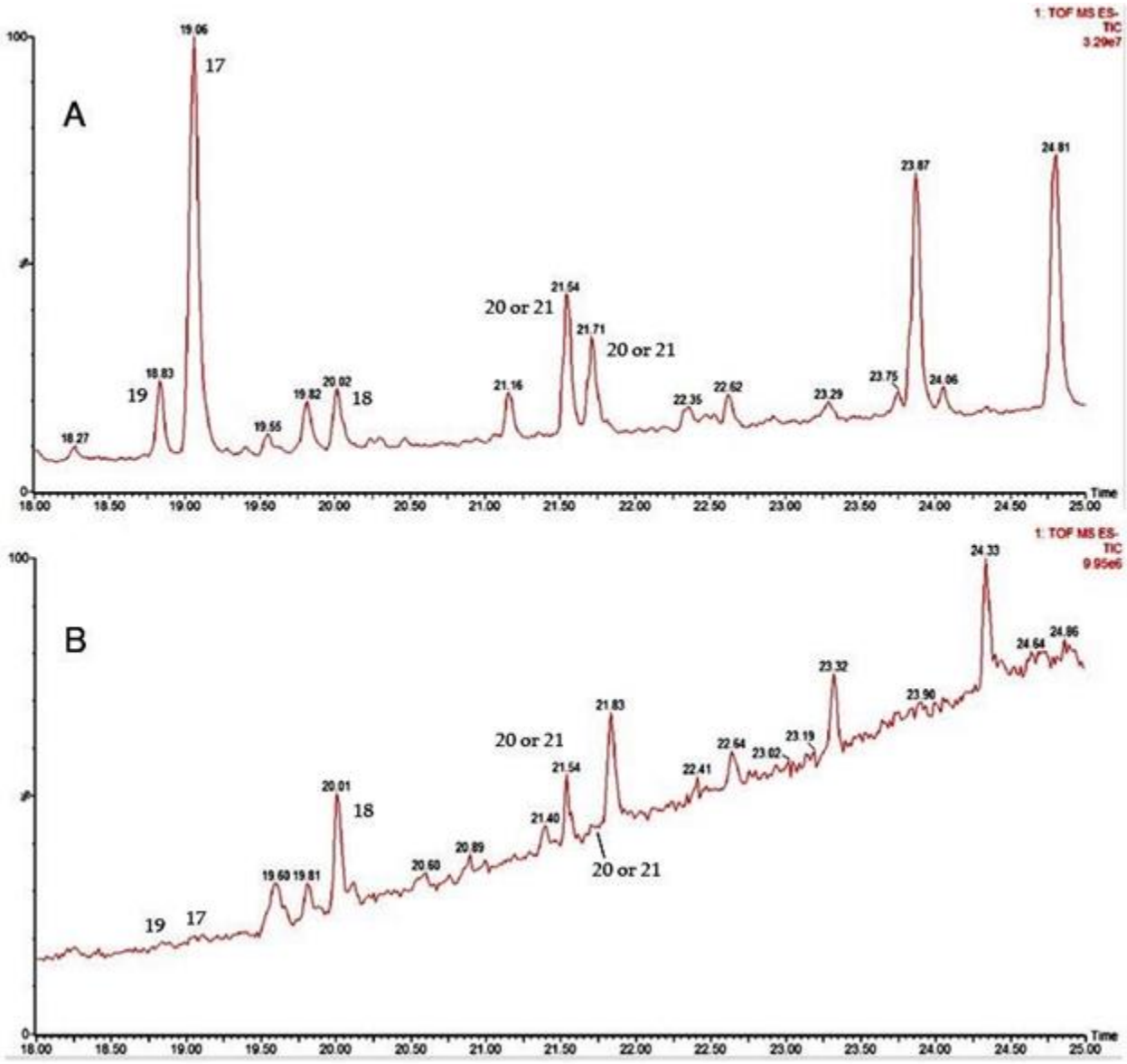


Figure S3
Total ion chromatogram of the ethanolic extract oleaginous fraction (OF) of Cuban *Piper aduncum* subsp. *ossanum* (A) and *Piper aduncum* subsp. *aduncum* (B)

Supplementary Materials No. 2
Identification based on accurate mass and fragmentation patterns of *Piper aduncum* subsp. *ossanum* and *P. aduncum* subsp. *aduncum* extracts
Table S1
Identification based on accurate mass and fragmentation patterns in the decoction of *Piper aduncum* subsp. *ossanum* and *P. aduncum* subsp. *aduncum*

Compound identification	MF	Rt (min)	Measured Mass (m/z) [M-H]-	Accuracy (ppm)	MS/MS Ions Negative ion mode	Reference	Ion relative abundance	
							Piper aduncum subsp. *aduncum*	*Piper aduncum* subsp. *ossanum*
(**16**) 8-(*C*-glucosyl)isorientin	$C_{27}H_{30}O_{16}$	7.04	609.1446	-1.6	**519.1158** (-90) **489.1050** (-120) **429.0836** (-90,-90) **399.0727** (-90,-120) **369.0621** (-120,-120)	-	+	+++
(**12**) Vicenin-2	$C_{27}H_{30}O_{15}$	7.70	593.1510	0.7	**503.1210** (-90) **473.1102** (-120) **413.0884** (-90,-90) **383.0779** (- 90,-120) **353.0669** (-120,-120)	-	+	+++
(**13**) 2"-(*O*-rhamnosyl)orientin/isoorientin	$C_{27}H_{30}O_{15}$	8.93	593.1505	-0.5	**473.1109** (-120) **447.0948** (-146) **429.0841** (-146,-18) **357.0620** (-146,-90) **327.0516** (-146,-120) **297.0402** (-150)	-	++	+++++
(**4**) Orientin*	$C_{21}H_{20}O_{11}$	8.93	447.0933	1.3	**429.0841** (-18) **357.0620** (-90) **327.0516** (-120) **297.0402** (-150)	21	+	+++++
(**5**) Isoorientin	$C_{21}H_{20}O_{11}$	9.10	447.0931	0.9	**429.0841** (-18) **357.0618** (-90) **327.0509** (-120) **297.0402** (-150) **285.0393** (-162)	-	+	++++
(**3**) 2"-(*O*-rhamnosyl)vitexin*	$C_{27}H_{30}O_{14}$	9.52	577.1559	0.3	**457.1158** (-120) **431.1107** (-146) **413.0892** (-146,-18) **341.0674** (-146,-90) **311.0565** (-146,-120) **293.0456** (-146,-18,-120)	21	+	++
(**14**) 8-(*C*-glucosyl)-4′-*O*-metyl-isovitexin or (**15**) 6-(*C*-glucosyl)-7-*O*-methylvitexin	$C_{28}H_{32}O_{15}$	9.52	607.1666	0.5	**517.1371** (-90) 487.1268 (-120) **397.0940** (-90,-120) **367.0836** (-120,-120)	-	+	++
(**1**) Vitexin*	$C_{21}H_{20}O_{10}$	9.62	431.0981	0.7	**413.0893** (-18) **341.0672** (-90)	21	+	++

					323.0564 (-90,-18) **311.0565** (-120) **283.0611** (-150)			
(**6**) Isoswertiajaponin* or (**7**) 4′-*O*-methylorientin*	$C_{22}H_{22}O_{11}$	10.23	461.1084	0.0	**371.0779** (-90) **341.0667** (-120) 311.0553 (-150)	21	+	++
(**2**) Isovitexin*	$C_{21}H_{20}O_{10}$	10.50	431.0980	0.5	**341.0674** (-90) 323.0566 (-90,-18) **311.0562** (-120) **283.0609** (-150)	20	+	++
(**6**) Isoswertiajaponin* or (**7**) 4′-*O*-methylorientin*	$C_{22}H_{22}O_{11}$	10.67	461.1084	0.0	**371.0774** (-90) **341.0675** (-120) 311.0555 (-150)	21	+	+
(**8**) 2″-(*O*-rhamnosyl)-4′-*O*-methylvitexin*	$C_{28}H_{32}O_{14}$	11.29	591.1718	0.7	**471.1317** (-120) **445.1135** (-146) **427.1052** (-146,-18) **367.0834** (-120,-104) **355.0831** (-146,-90) **337.0725** (164,-90) **325.0721** (-120,-146) **307.0615** (-164,-120) **283.0610** (-146,-162)	17, 21	+++++	+++++
(**9**) Cytisoside* or (**10**). swertisin*	$C_{22}H_{22}O_{10}$	11.72	445.1142	1.6	**355.0824** (-90) **337.0719** (-90, -18) **325.0720** (-120) **282.0533** (-164)	20, 21	+	++
(**9**) Cytisoside* or (**10**) swertisin*	$C_{22}H_{22}O_{10}$	13.18	445.1143	1.8	**355.0833** (-90) **337.0716** (-90, -18) **325.0720** (-120) **282.0534** (-164)	20, 21	+	+
Unknown	$C_{30}H_{33}O_{15}$	13.79	633.1819	0.0	441.1188, 381.0963, 339.0870, 307.0605	-	++	-
Unknown	$C_{12}H_{14}O_3$	17.18	205.0859	-2.9	330.9602, 294.9831	-	+	+++++

CL: Confidence level; *: Previously reported compound in *P. aduncum*; MS2 signal in bold: matching fragmentation patterns; MS2 ions signal underlined: matching patterns of flavonoid aglycones (Ag), namely, [Ag + 41]- and [Ag + 71]- for mono-C-glycosides and [Ag + 83]- and [Ag + 113]- for di-C-glycosides (Vukics & Guttman, 2010); Ion relative abundance (%): +++++ (80-100), ++++ (60-79), +++ (40-59), ++ (20-39), + (5-19), - not detected

Table S2
Identification based on accurate mass and fragmentation patterns in the ethanolic extract aqueous fraction (AF) of Cuban *Piper aduncum* subsp. *ossanum* and *P. aduncum* subsp. *aduncum*

Compound identification	MF	Rt (min)	Measured Mass (m/z) [M-H]-	Accuracy (ppm)	MS/MS Ions Negative ion mode	Reference	Ion relative abundance	
							Piper aduncum subsp. *aduncum*	*Piper aduncum* subsp. *ossanum*
(**16**) 8-(*C*-glucosyl)isorientin	$C_{27}H_{30}O_{16}$	7.04	609.1447	-1.5	**519.1156** (-90) **489.1053** (-120) **429.0846** (-90,-90) **399.0729** (-90,-120) **369.0620** (-120,-120)	-	+	++
(**12**) Vicenin-2	$C_{27}H_{30}O_{15}$	7.69	593.1505	-0.2	**503.1206** (-90) **473.1101** (-120) **413.0837** (-90,-90) **383.0776** (- 90,-120) **353.0668** (-120,-120)	-	++	++
(**13**) 2"-(*O*-rhamnosyl)orientin/isoorientin	$C_{27}H_{30}O_{15}$	8.91	593.1508	0.3	**447.0938** (-146) **357.0614** (-146,-90) **327.0506** (-146, 120) **297.0398** (-150)	-	++	+++++
(**4**) Orientin*	$C_{21}H_{20}O_{11}$	8.91	447.0933	1.3	**429.0836** (-18) **357.0614** (-90) **327.0506** (-120) **297.0398** (-150)	21	++	+++++
(**5**) Isoorientin	$C_{21}H_{20}O_{11}$	9.08	447.0924	-0.7	**429.0840** (-18) **357.0619** (-90) **327.0509** (-120) **297.0402** (-150) **285.0400** (-162)	-	+	++++
(**3**) 2"-(*O*-rhamnosyl)vitexin*	$C_{27}H_{30}O_{14}$	9.52	577.1558	0.2	**457.1150** (-120) **431.1016** (-146) **413.0888** (-146,-18) **341.0670** (-146,-90) **311.0559** (-146,-120) **293.0453** (-146,-18,-120)	21	++	++
(**14**) 8-(*C*-glucosyl)-4′-*O*-metyl-isovitexin or (**15**) 6-(*C*-glucosyl)-7-*O*-methylvitexin	$C_{28}H_{32}O_{15}$	9.52	607.1661	-0.3	**517.1381** (-90) **487.1253** (-120) **397.0936** (-90,-120) **367.0823** (-120,-120)	-	+	++
(**1**) Vitexin*	$C_{21}H_{20}O_{10}$	9.62	431.0983	1.2	**413.0893** (-18) **341.0672** (-90) **323.0564** (-90,-18) **311.0561** (-120) **283.0606** (-150)	21	+	+
(**6**) Isoswertiajaponin* or (**7**) 4′-*O*-methylorientin*	$C_{22}H_{22}O_{11}$	10.23	461.1082	-0.4	**371.0757** (-90) **341.0660** (-120) 311.0555 (-150)	21	+	++

(**2**) Isovitexin*	$C_{21}H_{20}O_{10}$	10.50	431.09876	-02	**341.0668** (-90) **323.0557** (-90,-18) **311.0559** (-120) **283.0605** (-150)	20	++	++
(**6**) Isoswertiajaponin* or (**7**) 4′-*O*-methylorientin*	$C_{22}H_{22}O_{11}$	10.67	461.1081	-0.7	**371.0765** (-90) **341.0663** (-120) 311.0540 (-150)	21	+	+
(**11**) Isospinosin*	$C_{28}H_{32}O_{15}$	10.97	607.1647	-2.6	**487.1257** (-120) **445.1111** (-164) **367.0803** (-120, -120) **325.0710** (-164, -120)	21	++	-
Unknown	$C_{30}H_{36}O_{17}$	11.03	667.1853	-3.1	621.1801, 607.1654, 501.1404, 459.1297, 339.0868	-	++	-
(**8**) 2″-(*O*-α-rhamnosyl)-4′-*O*-methylvitexin*	$C_{28}H_{32}O_{14}$	11.27	591.1703	-1.9	**471.1307** (-120) **445.1121** (-146) **427.1044** (-146,-18) 409.0940 (-146,-36) **367.0825** (-120,-104) **355.0788** (-146,-90) **337.0716** (164,-90) **325.0717** (-120,-146) **307.0610** (-164,-120) **283.0602** (-146,-162)	17, 21	+++++	++++
(**9**) Cytisoside* or (**10**) swertisin*	$C_{22}H_{22}O_{10}$	11.71	445.1135	0.0	**355.0845** (-90) **325.0710** (-120) **282.0519** (-164)	20, 21	++	++
(**9**) Cytisoside* or (**10**) swertisin*	$C_{22}H_{22}O_{10}$	13.17	445.1138	0.7	**355.0822** (-90) **325.0721**(-120) **282.0528** (-164	20, 21	+	+

CL: Confidence level; *: Previously reported compound in P. aduncum; MS2 signal in bold: matching fragmentation patterns; MS2 ions signal underlined: matching patterns of flavonoid aglycones (Ag), namely, [Ag + 41]- and [Ag + 71]- for mono-C-glycosides and [Ag + 83]- and [Ag + 113]- for di-C-glycosides (Vukics & Guttman, 2010); Ion relative abundance (%): +++++ (80-100), ++++ (60-79), +++ (40-59), ++ (20-39), + (5-19), - not detected

Table S3

Identification based on accurate mass and fragmentation patterns in the ethanolic extract oleaginous fraction (OF) of Cuban *Piper aduncum* subsp. *ossanum* and *P. aduncum* subsp. *aduncum*

Compound identification	**MF**	**Rt (min)**	**Measured Mass (m/z) [M-H]-**	**Accuracy (ppm)**	**MS/MS Ions Negative ion mode**	**Reference**	**Ion relative abundance**	
							Piper aduncum* subsp. *aduncum	***Piper aduncum* subsp. *ossanum***
(**19**) 4-hydroxy-3-(3-methyl-2-butenoyl)-5-(3-methyl-2-butenyl)-benzoic acid*	$C_{17}H_{20}O_4$	18.83	287.1281	-0.7	**243.1385, 188.0831**	39, 40	+	+
(**17**) Pinocembrin (5,7-dihydroxyflavanone)*	$C_{15}H_{12}O_4$	19.06	255.0656	-0.4	227.0703, **213.0545** 211.0752, 187.0746 185.0592,**171.0434**	31, 32	+	+++++
Unknown	$C_{17}H_{22}O_4$	19.82	289.1440	0.0	268.0380, 219.1021 175.1118	-	+	+
(**18**) Methyl-4-hydroxy-3-(3'-methyl-2'-butenyl)-benzoate*	$C_{13}H_{16}O_3$	20.02	219.1017	-1.8	**205.9616, 164.0460** 170.8705, **149.0229**	31, 39	++	+
Unknown	$C_{14}H_{18}O_3$	21.16	233.1172	-2.6	358.9914, 205.0858 178.0619, 160.0876	-	-	+
(**21**) 3-geranyl-4-hydroxybenzoic acid* or (**20**) nervogenic acid (methyl-2-butenyl-benzoic acid)*	$C_{17}H_{22}O_3$	21.54	273.1490	-0.1	**229.1588, 174.1035**	20, 39, 40, 41	+	++
(**21**) 3-geranyl-4-hydroxybenzoic acid* or (**20**) nervogenic acid (methyl-2-butenyl-benzoic acid)*	$C_{17}H_{22}O_3$	21.71	273.1488	-1.1	**229.1589, 174.1033**	20, 39, 40, 41	+	++
Unknown	$C_{18}H_{30}O_3$	21.84	293.2112	-1.7	281.08129, 195.1368	-	++	+
Unknown	$C_{18}H_{32}O_3$	22.62	295.2269	-1.4	431.9765, 397.0063 353.0152, 310.0636 287.1268, 255.0658	-	+	+
Unknown	$C_{32}H_{32}O_7$	23.87	527.2068	0.4	483.2167, 321.1127 271.1332, 255.0658	-	-	++++
Unknown	$C_{29}H_{30}O_7$	24.33	489.1904	-1.8	458.0810, 423.2141 395.9531, 257.9319	-	++	-
Unknown	$C_{32}H_{32}O_7$	24.81	527.2072	0.4	483.2198, 321.1132, 271.1335, 255.0661, 205.0858	-	-	++++
Unknown	$C_{34}H_{38}O_7$	25.51	557.2531	-1.4	253.0853, 210.0663, 205.0848	-	+	-