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Artigo

CHEMICAL COMPOSITION VARIABILITY IN THE *Uncaria tomentosa* (cat's claw) WILD POPULATION

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Uncaria tomentosa (cat's claw) is a vine widely distributed throughout the South-American rainforest. Many studies investigating the chemical composition of cat's claw have focused on the pentacyclic (POA) and tetracyclic oxindole alkaloids (TOA), quinovic acid glycosides (QAG), and polyphenols (PPH). Nevertheless, it is still uncertain how environmental factors affect chemical groups. The aim of this work was to better understand the influence of environmental factors (geographic origin, altitude, and season) on cat's claw chemical composition. Stem bark, branches and leaf samples were extracted and analyzed by HPLC-PDA. The data obtained were explored by multivariate analysis (HCA and PCA). Higher amounts of oxindole alkaloids and PPH were found in leaves, followed by stem bark and branches. No clear relationship was verified among geographic origin or altitude and chemical composition, which remained unchanged regardless of season (dry or rainy). However, three oxindole alkaloid chemotypes were clearly recognized: chemotype **I** (POA with *cis* D/E ring junction); chemotype **II** (POA with *trans* D/E ring junction); and chemotype **III** (TOA). Thus, environmental factors appear to have only a minor influence on the chemical heterogeneity of the cat's claw wild population. Nevertheless, the occurrence of different chemotypes based on alkaloid profiles seems to be clear.

Keywords: *Uncaria tomentosa*; environmental factors; chemical composition; oxindole alkaloids; chemotype.

INTRODUCTION

Uncaria tomentosa (Willd. ex Schult.) DC. (Rubiaceae) popularly known as cat's claw or "Uña de Gato", is a woody vine native to the Amazon rainforest and widely dispersed through other tropical areas of South and Central America.[1,2] Paired hook-like thorns rising from outstanding peduncles have originated its common name.[2] The cat's claw bark has been used in the Asháninka medicine for over two thousand years.[2] Hitherto the main bioactive compounds that were recognized are: (I) oxindole alkaloids, mainly tetracyclic (TOA) and pentacyclic oxindole alkaloids (POA);[1,2] (II) quinovic acid glycosides (QAG),[1,3] and (III) polyphenols (PPH) such as phenolic acids, flavonoids, and proanthocyanidins.[1,4]

Immunostimulant, antiviral, antitumor, anti-mutagenic, and anti-inflammatory activities have been ascribed to the pentacyclic oxindole alkaloids,[1,2,5] but also to flavonoids and proanthocyanidins,[6-8] and quinovic acid glycosides from cat's claw stem bark.[1,9] Owing to the still incipient status of cat's claw forest management, the stem bark is collected mostly from wild populations.[10,11] This would appear to explain the chemical heterogeneity often reported among samples from different geographic origin, climate conditions, and plant growth conditions.[2,10,12,13] A further explanation of this heterogeneity probably involves the influence of different types of extraction; including drying and separation procedures used in cat's claw extracts and derived products. Indeed, previous studies showed that cat's claw oxindole alkaloid isomerization can take place under mild process conditions such as those found in turbo-extraction, static maceration, and spray drying despite the very transient thermal effect.[14,15] Conversely, dynamic maceration and ultrasound-assisted extraction seem to be two techniques of choice aiming to preserve the original cat's claw oxindole alkaloid profile.[14]

Exploratory multivariate analyses, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), have been applied increasingly to establish relevant but sometime hidden relationships between variables and observations in rather complex systems, including plants.[16-18]

In that context, this study was designed to investigate whether geographic origin, altitude, and season qualitatively and quantitatively affect the profile of pentacyclic and tetracyclic oxindole alkaloids, polyphenols and quinovic acid glycosides in stem bark, leaves and branches from cat's claw wild samples.

EXPERIMENTAL

Collection sites of plant material

Twenty-two wild samples of *U. tomentosa* were collected in Peruvian rainforest, namely, Junín and Pasco (zone 1), Junín (zone 2), Ucayali (zone 3), and Loreto (zone 4) provinces (Figure 1 and Table 1S, supplementary material), in 2011 and 2012. Collection sites included altitudes from 121–1530 m were classified according to the FAO guidelines as eyebrow jungle (C, 3600–800 m), high jungle (H, 800–400 m), and low jungle (L, 400–80 m).[19] The GPS coordinates (latitude and longitude) of each collection site were accurately recorded. Stem bark, branches and leaves of each specimen were selected by hand. Stem bark samples were excised 1 m above de ground level and the stem diameter was measured. Nonetheless, their age could not be established accurately, because plant age and stem bark diameters are not correlated in cat's claw. The botanical identification of all wild samples was performed by J.R. Campos De la Cruz (Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos, Lima, Peru). Additionally, two commercial samples of cat's claw stem bark (SID and SQM) purchased from the Brazilian market were analyzed for comparison purposes.

Plant material drying and extraction

Plant material (stem bark, leaves and branches) was dried in an air-circulating convection oven (Memmert, Germany) at 40 ºC,

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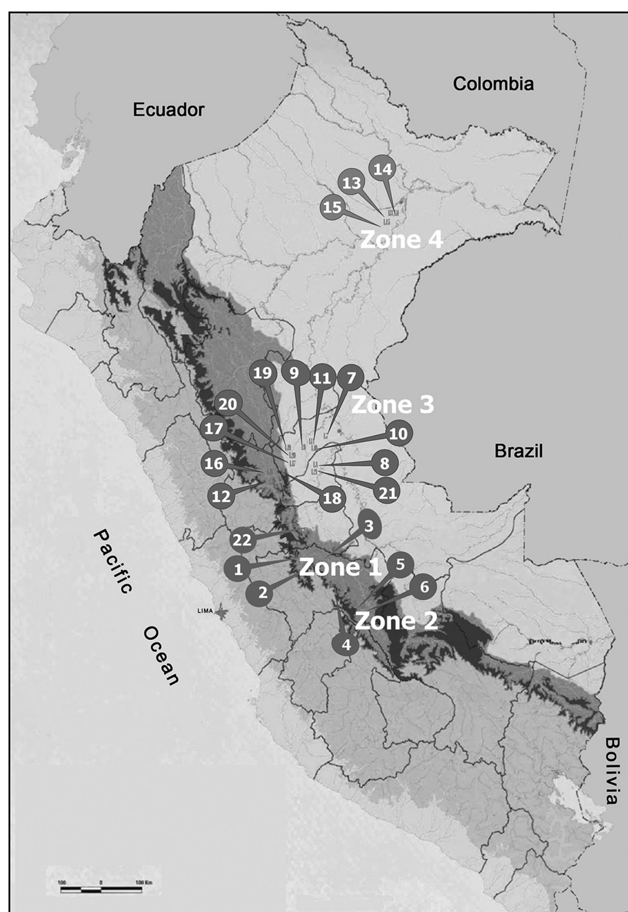

Figure 1. Collection sites of U. tomentosa wild samples in Peru: zone 1 (Junín and Pasco), zone 2 (Junín), zone 3 (Ucayali) and zone 4 (Loreto). For more details see Table 1S (Supplementary material)

and properly comminuted in a cutter mill (SK1 Retsch, Germany) provided with a 2 mm steel sieve. Powdered samples with a particle size ranging from 106–250 µm were selected for the extraction procedure.

One gram of each powdered sample was carefully weighed and extracted by 2h of dynamic maceration using a magnetic stirrer (300 rpm) (IKA RH basic 1, Germany) and 20 mL of hydroethanolic solution. Separately: polyphenols (PPH), oxindole alkaloids (POA and TOA) and quinovic acid glycosides (QAG) were extracted with 50, 63 and 69% (v/v) hydroethanolic solution, respectively.[20] The extractive solutions were filtered through a paper filter (Whatman, nº 2) and the filtrate volumes were reconstituted to their original value with the respective solvent. The samples for analysis were properly diluted and filtered through a 0.45 µm membrane (Millipore, USA) prior to injection.

HPLC-PDA analyses

All analyses were performed in an HPLC (Prominence,Shimadzu, Japan) equipped with an FCV-10 AL system controller, an LC-20 AT pump system, an SIL-20 A automatic injector and an SPD-M20A detector. All data were processed by LC-Solution Multi-PDA software. The results were expressed as g per 100 g of dry plant material (g%) by the mean value of three determinations.

Oxindole alkaloids

POA and TOA total content was assayed by a previously validated HPLC-PDA method[14] using mitraphylline (Phytolab, batch 2946, Germany) as external standard (LOD: 0.08 µg mL^{-1}; LOQ: 0.24 µg mL^{-1}). A Gemini-NX RP-18 column (250 x 4.6 mm i.d., 5 µm) (Phenomenex, USA) protected by an RP-18 guard column was employed. The mobile phase consisted of ammonium acetate buffer 10 mM (pH 7.0) (A) and acetonitrile (B) in a linear gradient program. The flow rate (1.0 mL/min) and temperature (23 ± 1 °C) were kept constant throughout the analysis. The detection was at 245 nm. POA and TOA contents were calculated by sum of individual alkaloid content, namely: speciophylline (SPEC), uncarine F (UNCF), pteropodine (PTER), isopteropodine (ISPTER), mitraphylline (MIT), isomitraphylline (ISMIT) for POA; rhynchophylline (RHY), isorhynchophylline (ISRHY) for TOA.

Quinovic acid glycosides

The QAG content was assayed by a previously validated HPLC-PDA method[3] using α-hederin (Extrasynthèse, batch 08040314, France) as external standard (LOD: 0.19 µg mL^{-1}; LOQ: 0.57 µg mL^{-1}). A Sinergy Fusion RP-18 column (150 x 4.6 mm i.d., 4 µm) (Phenomenex, USA) protected by an RP-18 guard column was employed. The mobile phase consisted of formic acid 0.01% (v/v) (A) and acetonitrile: formic acid 0.01% (90:10, v/v) (B) in a linear gradient program. The flow rate (1.0 mL/min) and temperature (35 ± 1 °C) were kept constant throughout the analysis. The detection was at 205 nm. The total content was calculated by sum of individual contents of the seven major peaks (Q1–Q7) previously characterized as quinovic acid glycosides by UV and MS-MS spectra.[3]

Polyphenols

The PPH content was assayed by a previously validated HPLC-PDA method[4] using chlorogenic acid (Fluka, batch 455159/1, Switzerland) (LOD: 0.38 µg mL^{-1}; LOQ: 1.14 µg mL^{-1}), caffeic acid (Extrasynthèse, batch 0381024, France) (LOD: 0.02 µg mL^{-1}; LOQ: 0.05 µg mL^{-1}) and rutin (Sigma, batch 128K1177, USA) (LOD: 0.44 µg mL^{-1}; LOQ: 1.35 µg mL^{-1}) as external standards. A Gemini RP-18 column (250 x 4.6 mm i.d., 5 µm) (Phenomenex, USA) protected by an RP-18 guard column was employed. The mobile phase consisted of trifluoroacetic acid 0.1% (v/v) (A) and methanol: trifluoroacetic acid (99.9:0.1, v/v) (B) in a linear gradient program. The flow rate (0.9 mL/min) and temperature (23 ± 1 °C) were kept constant. The detection was at 325 nm. The total content was calculated by sum of individual contents, namely: chlorogenic acid (COA), caffeic acid (CAA), rutin (RUT), peaks P1–P4 (Pavei *et al.*, 2010) and peaks P5–P6 previously characterized as flavonoids by UV spectra.[8]

Multivariate analysis

Hierarchical cluster (HCA) and principal component (PCA) analysis of chemical data were performed with the MINITAB® 15 software (State College, PA, USA). For HCA, the agglomerative hierarchical method was employed to join the clusters. Ward's linkage method was used to determine the distance between clusters and the Euclidean distance for their amalgamation. A correlation matrix was employed for PCA, and the number of components was determined from Kaiser's eigenvalue greater than 1.0 rule.[17]

RESULTS AND DISCUSSION

The study comprised 22 samples from wild specimens from four zones across the Peruvian Amazon rainforest. The collection sites were chosen considering a relatively narrow terrestrial longitude, but dissimilar altitudes. In general, the samples were collected in the rainy season (May and February), excepting samples 1 to 6, which were collected in the dry season (September). The extraction by dynamic

maceration applied in this work avoids any significant isomerization of cat's claw oxindole alkaloids, as reported earlier.[14,20]

Chemical composition

Stem bark

The total oxindole alkaloid (POA and TOA), polyphenols (PPH) and quinovic acid glycoside (QAG) contents in stem bark were dissimilar in wild (S1–S22) and commercial samples (SID and SQM) (Table 2S, supplementary material), evidencing the chemical heterogeneity of the specie.

The total oxindole alkaloid of wild samples varied from 0.328–2.591 g%, while the total POA content of wild and commercial samples ranged from 0.057–2.584 g% and 0.528–1.043 g%, respectively. Since all POA, namely speciophylline, uncarine F, pteropodine, isopteropodine, mitraphylline, and isomitraphylline, were detected in all samples, the variation in the POA content was mostly quantitative. In turn, TOA were detected only in 16 samples (S1–S3, S7–S9, S12–S20, and S22), and the total TOA content ranged from 0.001–1.036 g%. Although SID and SQM were declared as genuine samples of cat's claw bark by the suppliers, their alkaloid contents were markedly dissimilar from each other. Thus, while only POA were detected in SID, SQM showed significant contents of both POA and TOA, besides a total alkaloid content of about one-half that found in SID (0.597 and 1.043 g%, respectively). Differently to SQM, SID mets the USP-Monograph requirements regarding the maximum TOA (0.05 g%) and minimum POA (0.3 g%) contents, respectively.[21]

Regarding the QAG profile, all wild samples and both commercial ones showed seven peaks ascribed to QAG, as previously reported.[3] The total QAG content ranged from 0.047 to 0.796 g% in wild samples, while it was fairly invariable (0.169 g%) in both commercial ones. The peak Q3 (0.367–0.023 g%), previously characterized by MS-MS analysis as a monoglycosilated derivative with two 6-desoxy-hexose in sugar chain,[3] was preponderant in all wild samples evaluated.

The PPH content in wild samples ranged from 0.071–0.311 g%. The chlorogenic acid (0.018–0.235 g%) was the major polyphenol found in stem bark as previously mentioned.[6] Noteworthy, low but measurable rutin contents were detected in almost all samples analyzed, therefore precluding the use of this flavonoid as a chemical marker to detect adulteration with *Uncaria guianensis*, as earlier proposed.[22]

Branches

The chemical composition of cat's claw branches has been barely explored. The total oxindole alkaloid content in branches ranged from 0.347–1.431 g%, whereas POA and TOA contents ranged from 0.052–0.999 g% and 0.003–1.335 g%, respectively (Table 3S, supplementary material). In general, the total oxindole alkaloid content in branches was lower than that found in stem bark. Conversely, QAG were detected just as traces (0.004–0.034 g%) in some samples, mainly the peak Q4, a monoglycosilated derivative with one 6-desoxy-hexose in sugar.[3] The absence of significant QAG content seems to be a relevant feature of cat's claw branches compared to stem bark. Regarding PPH content (0.056–0.401 g%), the results were very similar to that found in stem bark, chlorogenic acid (0.020–0.255 g%) being the major compound. Differently from stem bark, the peak P2 (0.012–0.103 g%), previously characterized as a flavonol,[4] also appears as a prevalent polyphenol in cat's claw branches.

Leaves

The total oxindole alkaloid content in leaves was 0.360–4.792 g%, whereas POA and TOA content ranged from 0.041–2.193 g% and 0.003–4.371 g%, respectively (Table 4S, supplementary material). On the whole, the total oxindole alkaloid content in leaves was greater compared to stem bark and branches. These results are in accordance with a previous study, which described the accumulation of oxindole alkaloid in cat's claw leaves, mainly in young ones.[12]

Similarly as observed in stem bark and branches (Tables 2S and 3S, supplementary material), some samples (L6, L20, L21 and L22) have in common the fact that only POA were detected. In addition, mitraphylline and isomitraphylline were predominant in leaves samples L3, L5, L6, L8, and L10, while uncarine F, speciophylline, pteropodine and isopteropodine were the major alkaloids detected in L4, L11, L17, L19–L22. This finding appears to challenge the assertion that uncarine F and speciophylline are the predominant oxindole alkaloids in cat's claw leaves, mainly in young leaves.[12] It is worth mentioning that rhynchophylline and isorhynchophylline prevailed in samples L1, L2, L7, L9, L12-L16 and L18. Thus, these findings bring about a more intricate view of the oxindole alkaloid predominance in cat's claw leaves and its high chemical heterogeneity.

Similarly to that noticed in branches, the QAG content in branches, they were detected as traces in leaves from only five samples (Table 4S, supplementary material). In contrast, the PPH content in cat's claw leaves (0.160–0.630 g%) was significantly greater than that found in branches and stem barks. Significant amounts of chlorogenic acid (0.048–0.544 g%), peak P2 (0.012–0.103 g%) and rutin (0.006–0.114 g%) were found in all samples, while caffeic acid was detected just as traces.

Effect of altitude on the chemical composition

Stem bark

Some samples collected below 227 m, but also above 559 m, showed lower POA and TOA contents. Particularly POA-rich samples seem to grow preferably between 227–559 m above sea level (Figure 1Sa, supplementary material). This finding agrees with preceding data about the altitudinal effect on the alkaloid composition in cat's claw stem bark.[10] However, no further relationship was noticed between altitude and the alkaloid profile, either that of POA or TOA. Likewise, the total QAG content was uncorrelated with altitude of the collection site (Figure 1Sb, supplementary material). With regard to PPH, chlorogenic acid (Figure 1Sc, supplementary material) was prevalent in samples from lowlands below 121 m, but also in highlands above 613 m. This behavior seems to be the contrary of POA accumulation in stem bark samples. Nevertheless, no apparent relationship between chemical composition and altitude could be established clearly.

Branches

Both total and individual oxindole alkaloid contents in wild samples varied almost randomly with respect to the altitude of the collection site (Figure 2Sa, supplementary material). The TOA content was prevalent either at low or high altitudes and clearly exceeded the POA content in several samples, as observed in B1, B12, B15, B16, and B18. Conversely, high POA contents were identified in samples growing nearly above 230 m and in middle lands (227–347 m). As observed in stem bark wild samples, it was greater to the TOA content, except for sample B9. In relation to PPH, the chlorogenic acid content in branches was the highest in those samples collected either below 238 m or above 559 m (Figure 2Sb, supplementary material), while the peak P2 was distributed equally. Similarly to that observed in stem bark wild samples, POA and chlorogenic acid showed each other opposite distribution tendencies.

Leaves

The POA predominance was noticed in samples collected between 227–347 m, except for sample L9. However, a more detailed analysis

showed that the TOA content prevailed over POA in more than 50% of all leaves samples, especially in samples collected below 227 m (Figure 3Sa, supplementary material). This finding is consistent with the USP-Monograph requirement[21] of a maximum TOA content of 0.05 g% in cat's claw stem bark aiming to preclude any adulteration with cat's claw leaves.

As noticed before in stem bark and branches, chlorogenic acid was also the main polyphenol derivative found in leaves (Figure 3Sb, supplementary material). Thus, both leaves samples from lowlands below 238 m and highlands above 1080 m showed the highest chlorogenic acid content. Nonetheless, the data obtained are not conclusive enough to recognize a reliable relationship between polyphenol content and the altitude of the collection site.

Multivariate analysis of chemical composition

Stem bark

The analysis of HCA and PCA data ruled out any possible correlation among the chemical composition of stem bark wild samples, geographic origin and altitude (Figure 4Sa and 4Sc, supplementary material). In addition, POA (mitraphylline, isomitraphylline, speciophylline, uncarine F, pteropodine and isopteropodine) and TOA (rhyncophylline and isorhyncophylline) were located in opposite positions denoting an uncorrelated distribution. Similar behavior could be noticed in the case of POA-chlorogenic acid (Figure 4Sb and 4Sd, supplementary material). QAG and the majority of PPH were distributed among clusters almost randomically. A plausible explanation for this lack of correlation between chemical composition and the other variables considered here perhaps involves other factors, as the genetic one.

It is noteworthy the segregation of samples into two groups according to the alkaloid type – POA or TOA (Figure 4Sd, supplementary material), which becomes more intrincate when the oxindole alkaloids are analyzed separately. The analysis by HCA and PCA of POA and TOA showed three clusters emerging among stem bark samples (Figure 2), with about 86.5% of variability explained by PC1 and PC2 (Figure 2c and 2d).[16,17] The first cluster (group **I**) included the stem bark samples with predominance of POA *cis* D/E ring junction (pteropodine, isopteropodine, speciophylline and uncarine F). On the other hand, the second one (group **II**) was composed mainly by POA with *trans* D/E ring junction (mitraphylline and its isomer isomitraphylline), while the last cluster (group **III**) included only TOA (rhynchophylline and its isomer isorhynchophylline) (Figure 2b and 2d).

Remarkably, the three-cluster alkaloid pattern is repeated again in both the branches and the leaves of all wild samples analyzed (Figure 3b, 3d, 4b and 4d), depicting a more complex condition than that shaped by only two botanically equivalent chemotypes (tetracyclic or pentacyclic alkaloid-type), as proposed previously from cat's claw samples of root bark and leaves.[12] Given that the extraction procedure avoiding both POA and TOA isomerization was invariant throughout the work,[14,20] any interconversion due to the sample processing should be disregarded.

Samples of group I (S2–S4, S11, S17, S19–S22) and group **II** (S6, S5, S8, S10, S15) (Figure 2a and 2c), as well the commercial

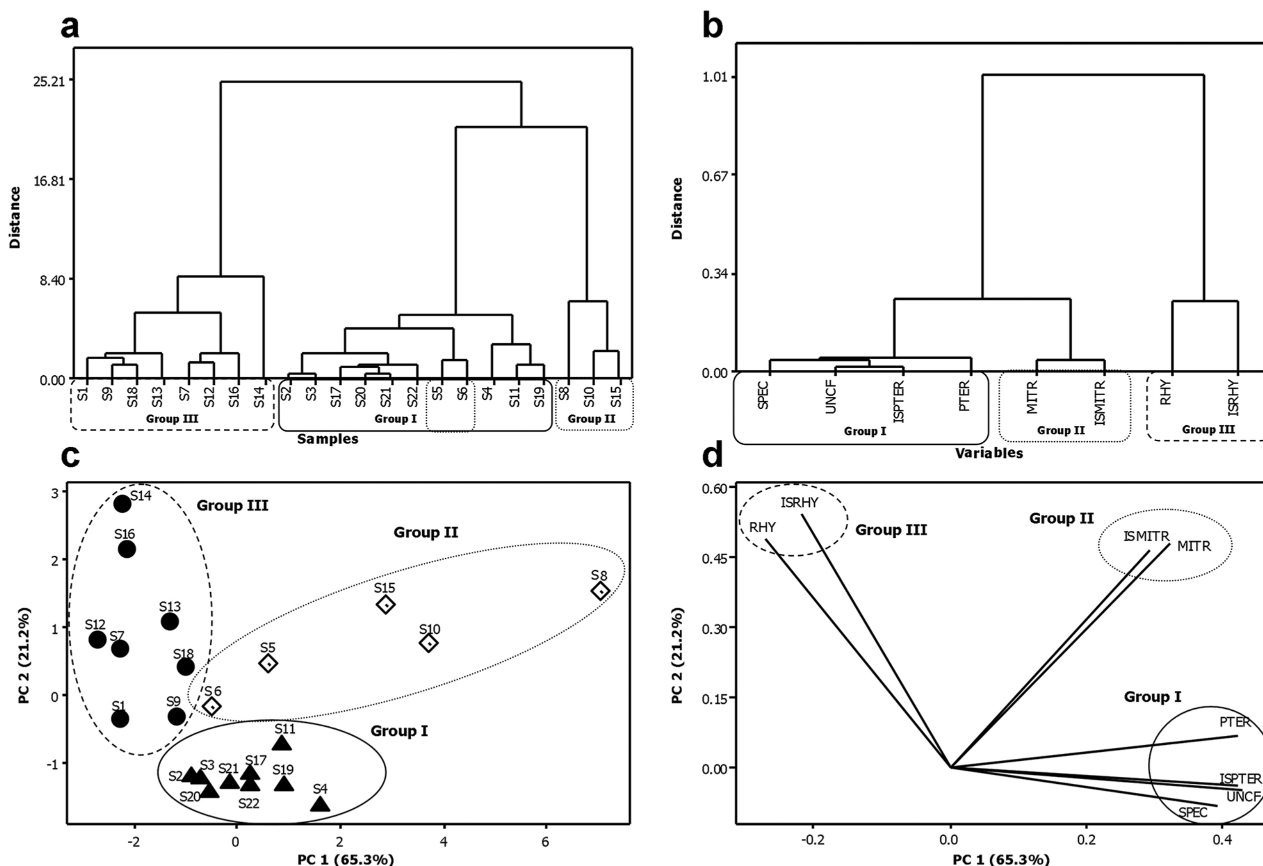

*Figure 2. HCA and PCA analyses of U. tomentosa stem bark samples considering only the oxindole alkaloids: **(a)** dendrogram of the samples using Ward's as linkage method and the Euclidean distance, **(b)** dendrogram of the variables using the single linkage method and correlation coefficient distance, **(c)** score and **(d)** loading plots (PC1 versus PC2) from the chemical data. Group I – predominance of POA with cis D/E ring junction, namely, speciophylline (SPEC), uncarine F (UNCF), pteropodine (PTER) and isopteropodine (ISPTER); Group II – predominance of POA with trans D/E ring junction, namely, mitraphylline (MITR) and isomitraphylline (ISMITR); Group III – predominance of TOA, namely, rhyncophylline (RHY) and isorhyncophylline (ISRHY)*

sample (SID), with POA contents higher than 0.3 g% (0.327–2.584 g%) but TOA content less than 0.05 g%, fulfilled pharmacopoeical requirements regarding cat's claw.[21] However, the samples of group **III** (S1, S7, S9, S12–S14, S16, S18) (Figure 2a and 2c) and commercial sample (SQM) showed TOA contents higher than 0.05 g% (0.069–1.036 g%). Therefore, they would be considered as a low quality raw material despite the fact all they were identified unambiguously as *U. tomentosa*. Althought some cat's claw activities have been ascribed to POA,[1,2] TOA exerted antihypertensive and neuroprotective activities.[23] Thus, pharmacopoeial constraint based on a TOA maximum content seems to be debatable, at least, despite its antagonistic immunological effect in relation to POA.[2]

Branches

After HCA and PCA results, the content of POA, TOA and polyphenols seems to be uncorrelated neither with the geographic origin nor with altitude of the collection site (Figure 5S, supplementary material). The QAG analyses were omitted because they occurred only as traces in almost all samples (Table 3S, supplementary material).

Again, the analyses of the oxindole alkaloids by HCA and PCA revealed three clearly defined clusters (groups **I**, **II** and **III**) (Figure 3). Briefly, the group **I** (POA with *cis* D/E ring junction) included the samples B4, B17, B19–B22; the group **II** (POA with *trans* D/E ring junction) the included the samples B3, B5, B6, B8, B10, B11; and the group **III** (TOA) composed by samples B1, B2, B7, B9, B12–B16, B18 (Figure 3a and 3c).

The cluster structure appears to be practically indifferent with respect to the plant part analyzed, ie, the oxindole alkaloid profiles remained unchanged qualitatively. Thus, the cluster pattern in cat's claw branches (Figure 3) was similar to that noticed in the stem bark (Figure 2). Compared to stem bark (Figure 2b and 2d), in the case of branches, the three clusters were still better defined, having a similar intensity of vectors and angle magnitudes in PCA (Figure 3d), and greater distance among clusters (groups **I**, **II** and **III**) after HCA (Figure 3b). Nonetheless, some exceptions are worth mentioning. For instance, stem bark samples 2 and 15 changed from groups **I** and **II**, respectively (Figure 2a and 2c), to the group **III**, when branches are considered (Figure 3a and 3c). Similarly, samples 3 and 11 displaced from group **I** to group **II**.

Leaves

The alkaloid composition was uncorrelated neither with geographic origin nor altitude, as former noticed in stem bark and branches (Figure 6S, supplementary material). The QAG are omitted since they were detected only as traces (Table 4S, supplementary material,). Again, the three-cluster pattern became evident (Figure 4b and 4d) still more clearly than before in branches (Figure 3b and 3d) and stem bark (Figure 2b and 2d). The POA with *cis* D/E ring junction (group **I**) included the samples L4, L11, L17, L19–L22; the POA with *trans* D/E ring junction (group **II**) included the samples L3, L5, L6, L8, L10; while TOA (group **III**) was composed by samples L1, L2, L7, L9, L12–L16, L18. The sample 11 present a rule exception moving from group **II** in the branches (Figure 3a and 3c) to the group **I** in leaves (Figure 4a and 4c).

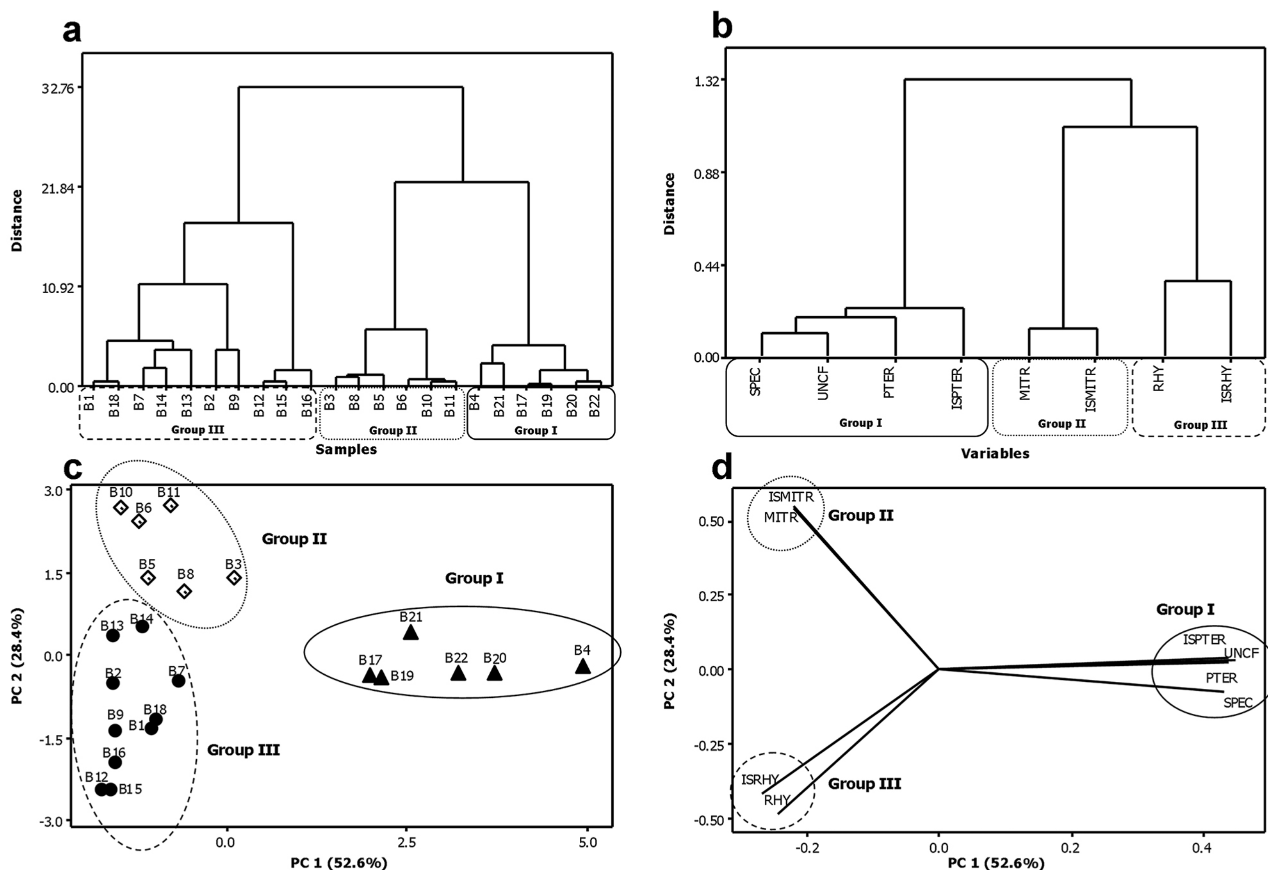

*Figure 3. HCA and PCA analyses of U. tomentosa branches samples considering only the oxindole alkaloids: **(a)** dendrogram of the samples using Ward's as linkage method and the Euclidean distance, **(b)** dendrogram of the variables using the single linkage method and correlation coefficient distance, **(c)** score and **(d)** loading plots (PC1 versus PC2) from the chemical data. Group I – predominance of POA with cis D/E ring junction, namely, speciophylline (SPEC), uncarine F (UNCF), pteropodine (PTER) and isopteropodine (ISPTER); Group II – predominance of POA with trans D/E ring junction, namely, mitraphylline (MITR) and isomitraphylline (ISMITR); Group III – predominance of TOA, namely, rhyncophylline (RHY) and isorhyncophylline (ISRHY)*

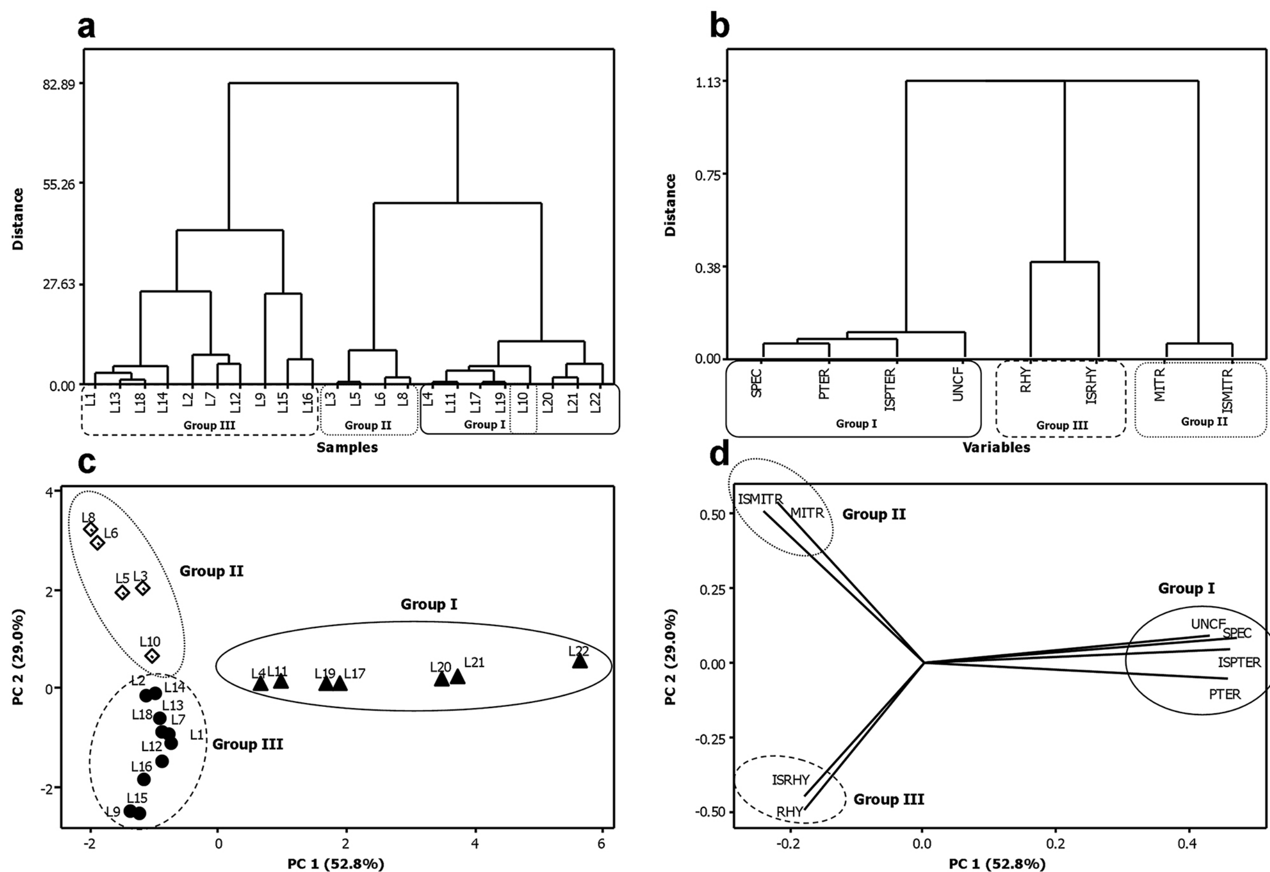

***Figure 4.** HCA and PCA analyses of U. tomentosa leaves samples considering only the oxindole alkaloids: **(a)** dendrogram of the samples using Ward's as linkage method and the Euclidean distance, **(b)** dendrogram of the variables using the single linkage method and correlation coefficient distance, **(c)** score and **(d)** loading plots (PC1 versus PC2) from the chemical data. Group I – predominance of POA with cis D/E ring junction, namely, speciophylline (SPEC), uncarine F (UNCF), pteropodine (PTER) and isopteropodine (ISPTER); Group II – predominance of POA with trans D/E ring junction, namely, mitraphylline (MITR) and isomitraphylline (ISMITR); Group III – predominance of TOA, namely, rhyncophylline (RHY) and isorhyncophylline (ISRHY)*

Influence of the season on the chemical composition of stem bark, branches and leaves

Cat's claw samples from group **I** (20 and 21), group **II** (8 and 10), and group **III** (16 and 18) comprising stem bark, branches and leaves were selected owing the alkaloid profile similarity among them. The samples were evaluated in the rainy season (February) and dry season (November) (Figure 5). The chemical profile of samples remained basically unchanged, although quantitative variations could be verified. Samples collected during the dry season showed higher PPH content than in the rainy season, especially for chlorogenic acid (Figure 5c). On the other hand, no accumulation in either the dry season or rainy season was found neither for oxindole alkaloids (POA and TOA) (Figure 5a) nor QAG (Figure 5b).

These results revealed that oxindole alkaloid profiles among the samples were consistently independent of season (Figure 5a). The finding stays in disagreement with a previous work, when the alkaloid profile from individual plants changed along different years and seasons.[24]

Chemotypes from cat's claw based on oxindole alkaloid profile

The chromatograms from samples 22, 6 and 12, illustrate the essential differences among samples belonging to group **I** (speciophylline, uncarine F, pteropodine, isopteropodine prevalence) (Figure 6a), group **II** (mitraphylline and isomitraphylline prevalence) (Figure 6b), and group **III** (rhynchophylline and isorhynchophylline prevalence) (Figure 6c). As demonstrated by the multivariate analysis, the alkaloid profile became more specific as follows: stem bark → branches → leaves. Taking into account that the biosynthesis of cat's claw alkaloids occurs in leaves[12] the diversification of the alkaloid profile appears to occur as it moves downward in the plant. This can be related to the bioconversion of D/E *trans* ring junction to D/E *cis* ring junction and vice versa.[12,25]

No less important is the fact the oxindole alkaloid profile of samples 2 and 15 (Figure 7) was quite different from that found in other either wild samples. Also, in both samples the TOA predominated over POA in both leaves and branches. Probably, the bioconversion of TOA to POA occurs from leaves → branches → stem barks as demonstrated from leaves of cat's claw micropropagated plantlets at different growth stages.[13]

CONCLUSIONS

Cat's claw wild samples showed a highly heterogeneous chemical constitution especially for oxindole alkaloids. The highest alkaloid and polyphenol contents were found in leaves followed by stem bark and branches, while the quinovic acid glycosides were detected in significant amounts only in the stem barks. Although any relationship between chemical composition and environmental factors (geographic origin and altitude) could be assessed, three chemotypes (chemotype **I** – POA with *cis* D/E ring junction; chemotype **II** – POA with *trans* D/E ring junction; chemotype **III** – TOA) with specific alkaloid profiles were clearly verified. Thus, the evaluation and definition of

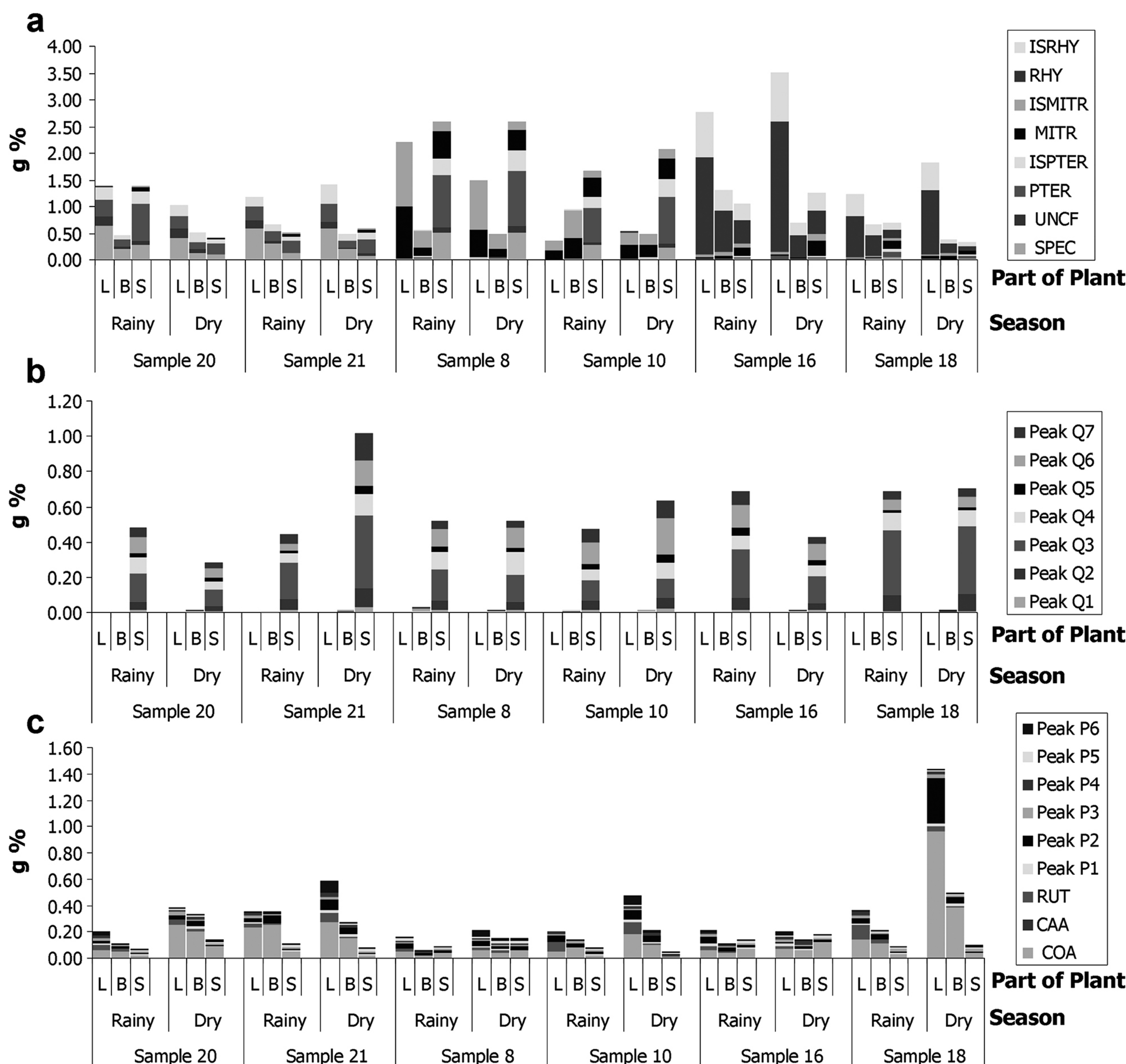

***Figure 5.** Chemical variation between rainy season (February/2012) and dry season (November/2012) in (S) stem bark, (B) branches and (L) leaves of U. tomentosa wild samples from group I (20 and 21), group II (8 and 10), and group III (16 and 18): **(a)** oxindole alkaloids (pentacyclic (POA): mitraphylline (MITR), isomitraphylline (ISMITR), speciophylline (SPEC), uncarine F (UNCF), pteropodine (PTER) and isopteropodine (ISPTER); tetracyclic (TOA): rhyncophylline (RHY) and isorhyncophylline (ISRHY)), **(b)** quinovic acid glycosides (QAG: peaks Q1−Q7), and **(c)** polyphenols (PPH: chlorogenic acid (COA), caffeic acid (CAA), rutin (RUT), peaks P1−P6)*

an alkaloid profile in cat's claw seems to be essential, since it can alter the response mainly for biological activities ascribed to the oxindole alkaloids.

SUPPLEMENTARY MATERIAL

Available at http://quimicanova.sbq.org.br, as a PDF file, with free access. The altitude effect on chemical composition of stem bark, branches and cat's claw leaves are shown in Figures 1S-3S, respectively. HCA and PCA analysis obtained from total matrix analysis of stem bark, branches and cat's claw leaves are shown in Figures 4S-6S, respectively. Detailed description about origin of cat's claw wild samples are shown in Table 1S. The results of chemical composition analysis obtained from samples of stem bark, branches and cat's claw leaves are shown in Tables 2S-4S, respectively.

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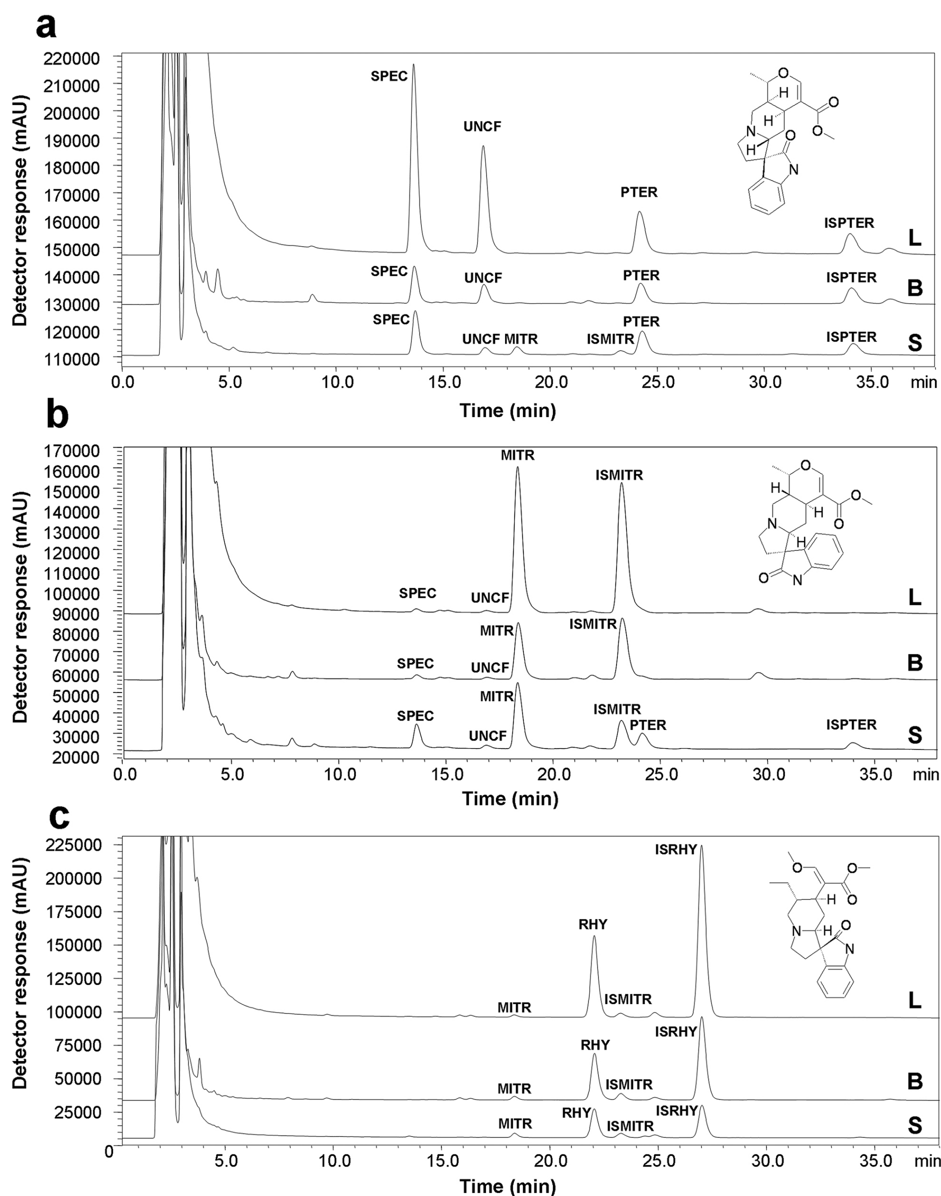

*Figure 6. HPLC-PDA chromatograms of oxindole alkaloids in (S) stem bark, (B) branches and (L) leaves from U. tomentosa wild samples: **(a)** sample 22 (group I – predominance of POA with cis D/E ring junction, namely, speciophylline (SPEC), uncarine F (UNCF), pteropodine (PTER) and isopteropodine (ISPTER)), **(b)** sample 6 (group II – predominance of POA with trans D/E ring junction, namely, mitraphylline (MITR) and isomitraphylline (ISMITR)) and **(c)** sample 12 (group III – predominance of TOA, namely, rhyncophylline (RHY) and isorhyncophylline (ISRHY)). Detection at 245nm*

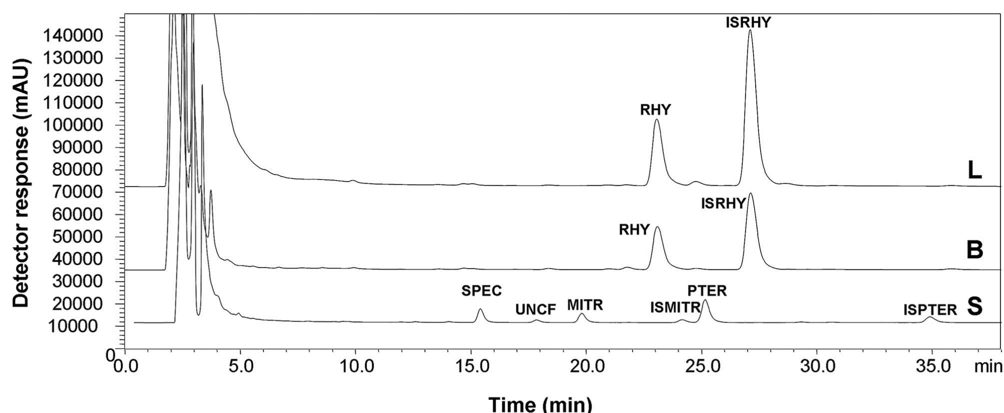

Figure 7. HPLC-PDA chromatograms of oxindole alkaloids in (S) stem bark, (B) branches and (L) leaves from U. tomentosa sample 2. Speciophylline (SPEC), uncarine F (UNCF), pteropodine (PTER), isopteropodine (ISPTER), mitraphylline (MITR) isomitraphylline (ISMITR), rhyncophylline (RHY) and isorhynco-phylline (ISRHY). Detection at 245nm

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