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RESEARCH PAPER

Quantification and characterization of alkaloids from roots of *Rauwolfia serpentina* using ultra-high performance liquid chromatography-photo diode array-mass spectrometry

Satyanarayanaraju Sagi[1] · **Bharathi Avula**[1] · **Yan-Hong Wang**[1] · **Ikhlas A. Khan**[1,2,3]

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Abstract A new UHPLC-UV method has been developed for the simultaneous analysis of seven alkaloids [ajmaline (**1**), yohimbine (**2**), corynanthine (**3**), ajmalicine (**4**), serpentine (**5**), serpentinine (**6**), and reserpine (**7**)] from the root samples of *Rauwolfia serpentina* (L.) Benth. ex Kurz. The chromatographic separation was achieved using a reversed phase C_{18} column with a mobile phase of water and acetonitrile, both containing 0.05 % formic acid. The seven compounds were completely separated within 8 min at a flow rate of 0.2 mL/min with a 2-μL injection volume. The method is validated for linearity, accuracy, repeatability, limits of detection (LOD), and limits of quantification (LOQ). Seven plant samples and 21 dietary supplements claiming to contain *Rauwolfia* roots were analyzed and content of total alkaloids (**1**–**7**) varied, namely, 1.57–12.1 mg/g dry plant material and 0.0–4.5 mg/day, respectively. The results indicated that commercial products are of variable quality. The developed analytical method is simple, economic, fast, and suitable for quality control analysis of *Rauwolfia* samples and commercial products. The UHPLC-QToF-mass spectrometry with electrospray ionization (ESI) interface method is described for the confirmation and characterization of alkaloids from plant samples. This method involved the detection of $[M+H]^+$ or M^+ ions in the positive mode.

Keywords *Rauwolfia serpentina* · Alkaloids · Ultra-high performance liquid chromatography-photo diode array · Ultra-high performance liquid chromatography-quadrupole time of flight-mass spectrometry · Dietary supplements

Introduction

Rauwolfia is a genus of plants in the dogbane family (Apocynaceae), with 110 species of shrubs and trees native to tropical areas of the world (http://www.britannica.com/EBchecked/topic/492199/Rauvolfia). It is a rich source of secondary metabolites such as indole alkaloids [1]. The roots of *Rauwolfia serpentina* (L.) Benth. ex Kurz have been used in native Indian medicine for treatment of various illnesses [2] and have been mainly used to treat hypertension [3–5]. The major alkaloids from the *R. serpentina* are reserpine, ajmaline, serpentine, serpentinine, ajmalicine, and yohimbine [6–8]. The chemical constituents of *Rauwolfia* varied based on their species [9] and plant parts [10]. Roots of *R. serpentina* and *Rauwolfia vomitoria* Afzel are the two important plants produces therapeutically useful alkaloids. *R. vomitoria* contains similar chemical constituents as that of *R. serpentina.* According to reported literature, *R. vomitoria* contains twice as much reserpine than the *R. serpentina* plant of India [6, 9, 11, 12]. Reserpine is a potent substance which shared both central nervous system depressant and hypotensive actions [2]. It is the most common alkaloid in many species of *Rauwolfia* [3] and found to cause toxicity even at doses as small as 0.25 mg/day [13]. There is a need to develop a suitable method to find the levels of reserpine and

✉ Ikhlas A. Khan
ikhan@olemiss.edu

[1] National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, 1558 University Circle, University, MS 38677, USA

[2] Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

[3] Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

also use to identify the chemical fingerprint profiles of *R. serpentina* from dietary supplements.

Numerous methods have been reported in literature for both qualitative and quantitative analyses of *Rauwolfia* alkaloids which includes TLC [14, 15], HPTLC [16–19], HPLC [12, 20, 21], CE-MS [22], DART-MS [23], GC-MS [24], and LC-QTof-MS [25]. Court et al. [14] worked on different TLC systems for the separation of 12 *Rauwolfia* alkaloids on silica gel. Separations and retention behavior of *Rauwolfa*, corynanthe, and pseudocinchona alkaloids on unmodified silica gel was studied by Xuan et al. [15]. The separation of six indole alkaloids by HPLC and HPTLC was studied by Klyushnichenko et al. [16]. Analysis of 12 alkaloids in three *Rauwolfia* species: *R. serpentina*, *R. vomitoria*, and *Rauwolfia canescens* by HPLC with fluorescence detection was described by Robinson [12]. Some of the researchers used one or more techniques like TLC, HPTLC, and HPLC to develop a fingerprint alkaloidal profile for *R. serpentina* and determination of reserpine content [17–19]. Cieri [20] described a LC method for determination of reserpine and rescinnamine in *R. serpentina* commercial powders and tablets. The combined content of reserpine and rescinnamine in powders and tablets is 0.13–0.16 and 0.12–0.16 %, respectively. Gerasimenko et al. [21] developed an efficient system for the separation of 22 indole alkaloids by HPLC and its application for the analysis of alkaloids in inter-generic somatic hybrid cell cultures of *R. serpentina*×*Rhazya stricta*. Analysis of alkaloids from roots of *R. serpentina* by using CE-MS [22] and analysis of vomilenine and reserpine by using DART-MS [23] were reported in the literature. Preparative layer chromatographic [26], UV-visible spectrophotometric [27], HPTLC [28], HPLC [29], HPLC, and GC-MS [24] methods have been reported for analysis of alkaloids from other *Rauwolfia* species. Hong et al. demonstrated the screening and identification of many of the compounds present in *R. verticillata* by using HPLC-QToF-MS [25]. The limitations of this method are 60 min run time and sample extraction was carried under reflux at 70 °C for 2 h, no quantification, and no applicability of method for real samples. Recently, Kumar et al. [30] reported a DART-MS combined with principal component analysis for rapid fingerprinting of *Rauwolfia* species and their discrimination. DART-MS methods are rapid for screening of known compounds, and the major limitation was identification of compounds having same molecular weight or isomeric compounds. Karioti et al. [31] demonstrated a combined HPLC-DAD-MS, HPLC-MS^n, and NMR spectroscopy for quality control of several plant extracts that include *Rauwolfia*. Complexity of this method is use of HPLC-MS and NMR and run time of 35 min and able to identify only four alkaloids. Limits of detection of alkaloids in the above mentioned methods was found to be in the range 0.78–6.0 μg/mL (ajmaline), 0.05–4.0 μg/mL (ajmalicine), 0.2–1.3 μg/mL (yohimbine), and 0.39–8.0 μg/mL (reserpine) for 10 μL injection volumes.

All these methods have either long run times, or no quantitative analysis, or lack of sensitivity, or poor validation data. In this work, (i) quantitative analysis using UHPLC-PDA for seven indole alkaloids was developed for six samples of *R. serpentina*, one sample of *R. vomitoria* and 21 dietary supplements. (ii) Structural characteristics of indole alkaloids from the methanolic extracts of dried roots of *R. serpentina* have been also investigated using UHPLC-QToF-MS/MS in positive ion mode. This method involved the use of MS/MS characterization of alkaloids and fragmentation of seven reference compounds. The fragmentation patterns of seven reference compounds and other alkaloids from the extracts were identified or tentatively characterized on the basis of retention times, accurate mass, and fragmentation patterns.

Experimental

Chemicals and standards

The reference standards ajmaline (**1**), and ajmalicine (**4**) were purchased from Indofine Chemical Company, Hillsborough, NJ, USA. Coryanthanine (**3**) was purchased from Chromadex, Irvine, CA, USA. Yohimbine (**3**) and reserpine (**7**) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Serpentine (**5**) and serpentinine (**6**) were purchased from Quality Phytochemicals LLC, East Brunswick, NJ, USA. Purity of reference standards is greater than 96 %. Chemical structures of all the reference standards are shown in Fig. 1. The identity and purity of these compounds was confirmed by chromatographic and spectral data (UV and MS). All the solvents, acetonitrile, methanol, and formic acid used are of HPLC certified grade obtained from Fisher Scientific, USA. Water for the mobile phase was purified using a Milli-Q system (Millipore).

Plant materials

Botanical Reference Material roots of *R. serpentina* L Benth. ex Kurz (#BRM 15898) was purchased Chromadex, Irvine, CA, USA, and dried roots of *R. serpentina* (#AU 7535) was obtained from Center for Research in Indian Systems of Medicine (CRISM), India. Roots of *R. serpentina* (#CS 1623, #CS 1750, #CS 15949, and #CS 16968) were purchased commercially. Fourteen dietary supplements of powder (# DS 15929), capsules (DS 15933, DS 15937–38, DS 15940–42, DS 15945, DS 15947–48, DS 15950), tablets (# DS 15931–32, DS 15943), liquid (#DS 15935), and seven pre-work out supplements powders of (# DS 15928, DS 17008, DS 17016, DS 17019, and DS 17027) and capsule (# DS 17028) claiming to contain *R. serpentina* or *R. vomitoria* were purchased online. Specimens of all samples are deposited at the National Center for Natural Products Research's

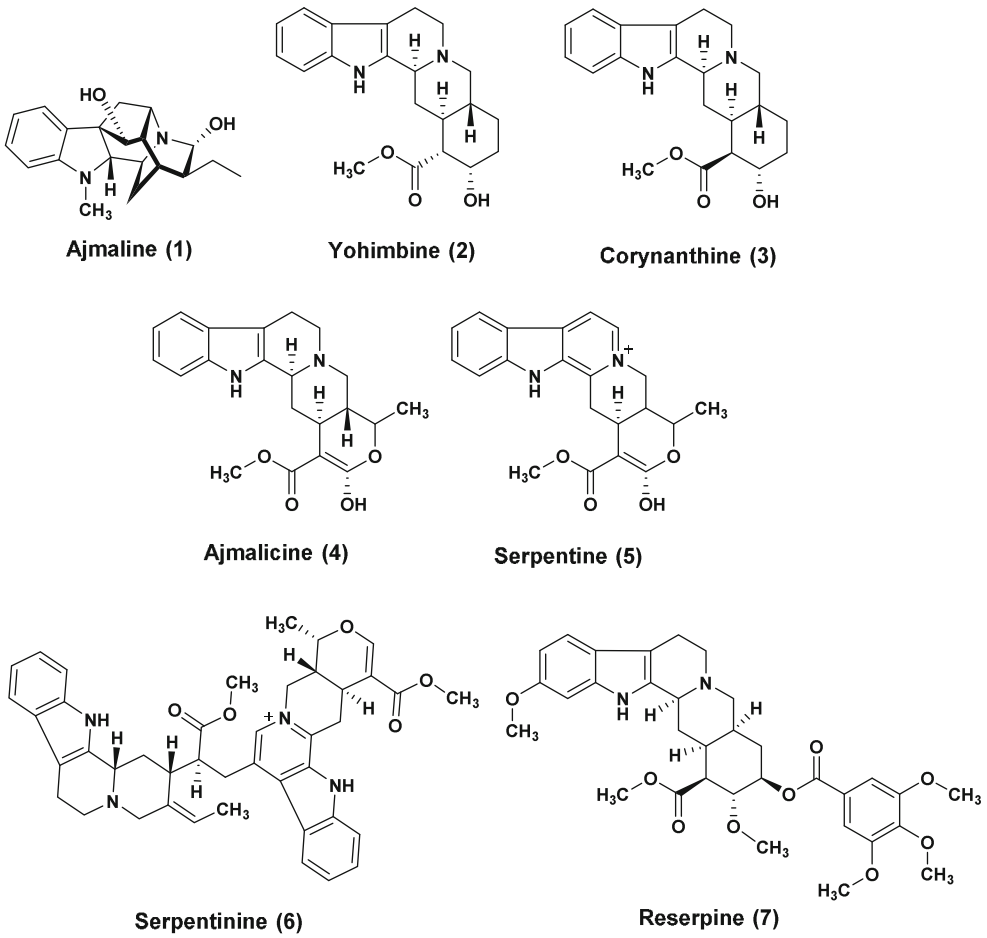

Fig. 1 Chemical structure of seven indole alkaloids

(NCNPR) botanical repository, The University of Mississippi, University, Mississippi, USA.

Preparation of plant samples and products

Dry plant samples (0.5 g) were taken in centrifuge tube, add 2.0 mL of 2 % formic acid in methanol, then sonicated for 30 min followed by centrifugation for 10 min at 4000 rpm. The clear supernatant was collected in a 10-mL volumetric flask. This procedure was repeated four more times; the volume of the pooled supernatant liquid in the volumetric flask was adjusted to 10 mL with 2 % formic acid in methanol. This solution was filtered through 0.45 μm syringe filter into a 2-mL vial. For liquid products, 1.0 mL was taken and directly filtered through 0.45 μm syringe filter and solution used for analysis.

The commercial products analyzed in this work were in two forms including: solids (powders/capsules/tablets and liquids). Five tablets were weighed and then pulverized with a mortar and pestle. For capsules, five samples were weighed, opened, and the contents were emptied, then mixed and triturated in a mortar and pestle. An adequate amount of capsule contents or tablets were weighed (average weight of dosage form), and the same extraction procedure was followed as for plant samples.

Preparation of stock and working standard solutions

Accurately weighed amounts of the individual reference compounds were dissolved in methanol to obtain 1.0 mg/mL. A solution of mixture of seven reference compounds was prepared by mixing the appropriate amounts of each individual standard stock solution in methanol. The concentrations of the reference standards used for construction of calibration curve UHPLC-PDA method were in range of 1–100 μg/mL.

Ultra-high performance liquid chromatography-PDA(UHPLC-PDA)

All analyses were performed with a Waters Acquity UPLC™ system (Waters Corp.) that included a binary solvent manager, sampler manager, column compartment, and PDA (Waters Acquity model code UPD) controlled by a Waters Empower 2 data station. An Acquity UPLC BEH Shield RP18 (1.7 μm, 50×2.1 mm) also from Waters was used. The column and sample temperatures were maintained at 40 and 15 °C,

respectively. The column was equipped with a guard column (Vanguard 2.1×5 mm; Waters Corp.). The mobile phase consisted of water (0.05 % formic acid) (A) and acetonitrile (0.05 % formic acid) (B) at a flow rate of 0.2 mL/min, which were used with the following gradient: 0 min, 15 %B; 3 min, 15 %B; 4 min, 20 %B; 6 min, 30 %B; 8 min, 50 %B; 10 min, 100 %B. The analysis was followed by a 3-min washing procedure with 100 % B and a re-equilibration period of 3.5 min. A strong needle wash solution (95:5, acetonitrile/water) and weak needle wash solution (10:90, acetonitrile/water) were used. The run time for analysis was 10 min. Aliquot of 2 μL was used for injection volume. The detection of analytes was done at different wavelengths.

Ultra-high performance liquid chromatography-mass spectrometry (UHPLC/QToF-MS)

The liquid chromatographic system was an Agilent Series 1290 comprised of the following modular components: binary pump, a vacuum solvent degasser, an autosampler with 108-vial well-plate trays, and a thermostatically controlled column compartment (Agilent Technologies, Santa Clara, CA, USA). Separation was achieved on an Agilent Poroshell 120 EC-C_{18} (2.1×150 mm, 2.7 μm) column. The mobile phase consisted of water with 0.1 % formic acid (A) and acetonitrile with 0.1 % formic acid (B) at a flow rate of 0.23 mL/min, with gradient elution of 0.0 min, 15 %B; 20.0 min, 30 %B; 25.0 min, 100 %B. Each run was followed by a 4-min wash with 100 % B and an equilibration period of 5 min with 15 % B. Two microliters of sample was injected. The column temperature was set at 40 °C. The mass spectrometric analysis was performed with a QToF-MS/MS (Model #G6530A, Agilent Technologies, Santa Clara, CA, USA) equipped with an ESI source with Jet Stream technology using the following parameters: drying gas (N_2) flow rate, 9.0 L/min; drying gas temperature, 250 °C; nebulizer, 35 psig; sheath gas temperature, 325 °C; sheath gas flow, 10 L/min; capillary, 3000 V; skimmer, 65 V; Oct RF V, 750 V; fragmentor voltage, 125 V. The acquisition was controlled by Agilent MassHunter Acquisition Software Ver. A.05.01, and the data were processed with MassHunter Qualitative software Ver. B.06.00. Standards were analyzed using targeted MS/MS where the quadrupole was set to an isolation width of 1.3 *m/z* for the precursor of each standard. MS/MS spectra were collected with collision offset voltages of 0, 10, 20, 30, and 40 V for reference standards. Accurate mass measurements were obtained by means of reference ion correction using reference masses at *m/z* 121.0509 (protonated purine) and 922.0098 [protonated hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine or HP-921] in positive ion mode. The compounds were confirmed in each spectrum. For this purpose, the reference solution was introduced into the ESI source via a T-junction using an Agilent Series 1200 isocratic pump (Agilent Technologies, Santa Clara, CA, USA) with 100:1 splitter set at a flow rate of 20 μL/min.

Validation

The newly developed UHPLC-PDA was validated in terms of linearity, LOD, LOQ, precision, accuracy, stability, system suitability, and robustness according to ICH guidelines [32].

Linearity of the method was determined by plotting calibration curves of the all seven reference compounds at seven different concentration levels. Calibration curves were plotted using concentration of reference standards vs peak area. The regression equation and correlation coefficients were obtained by least square regression analysis. LOD and LOQ were determined by measuring signal-to-noise of the analytes at lower concentration and were defined as the signal-to-noise ratio equal to 3:1 and 10:1, respectively.

Intra- and inter-day precision of the method was evaluated by analyzing samples #BRM 15898. Nine replicates (about 500 mg) of #BRM 15898 were weighed and extracted three times a day for three consecutive days using the previously mentioned extraction procedure. The extracted samples were analyzed using the developed UHPLC-PDA method. The accuracy of the method was determined by analyzing the same samples. After five exhaustive extractions of these samples, the material was dried. Known amounts of reference standards at two concentration levels were added to these samples, extracted, and analyzed using developed method. The stability of the analytes was evaluated by analyzing the reference standards and samples stored under refrigeration conditions at 4 °C for 24, 48, and 72 h. Room temperature stability of reference standards and samples were tested for 24 h. System suitability test was carried out by injecting 2 μL of mixture of standard solution at least six times. Robustness was carried out by making small changes to the method conditions, and their results were evaluated.

Results and discussion

Method development

UHPLC-PDA

Alkaloids present in *Rauwolfia* species are having different acid–base behavior. According to Lucas [7], they are classified into three types: strong, moderately, and weakly basic. The selected reference standards in this study fall in all three categories. Examples: serpentine is strongly basic, ajmaline is moderately basic whereas yohimbine, ajmalicine, and reserpine are weakly basic. Due to the difference in acid–base

behavior separation system that is satisfactory for the strongly basic alkaloids may be unsatisfactory for the weakly basic alkaloids. Various combinations of solvents with varying polarities were tested for achieving good separation and peak shape. Optimum chromatographic separation of the reference standards was achieved on Acquity UPLC™ BEH Shield RP18 (1.7 μm, 50×2.1 mm) using water (A) and acetonitrile (B) both containing 0.05 % formic acid. The seven reference compounds showed different UV absorption spectra and based on the UV absorption maxima detection wavelengths were selected as 245 nm for compound **1**, 272 nm for compounds **2**–**3**; 249 nm for compounds **4**–**5**, and 256 and 268 nm for compounds **6** and **7**, respectively.

UHPLC-QToF-MS

Agilent Poroshell 120 EC-C_{18} (2.1×150 mm, 2.7 μm) column was used to achieve an optimum separation of the standard reference compounds. The mobile phase consisted of water with 0.1 % formic acid (A) and acetonitrile with 0.1 % formic acid (B) at a flow rate of 0.23 mL/min with gradient elution was used. Samples were analyzed by UHPLC-QToF-MS in all-ion MS-MS mode where experiment 1, Q1 scans *m/z* 100–1300 and transmitting the ions through collision cell with low collision offset voltage (0 V). These ions are then pushed into QToF to acquire exact mass and experiment 2 with collision offset voltage (30 V) and to fragment all the ions transmitted by Q1 and exact mass measurements of all the fragment ions are acquired by the QToF analyzer. Figure 2 shows an example of mass spectra at low and high collision offset voltages respectively for reserpine. The accurate mass data, MS fragmentation, and retention time of each reference standards were used to identify these compounds in the *R. serpentina*, *R. vomitoria* extracts, and commercial products.

Extraction optimization

Two different extraction methods via sonication and alkaloidal extraction were tired, and results (not presented) are more favorable to sonication extraction. Extraction optimization was carried out in terms of solvent via methanol, acidified methanol with formic acid (FA), and aqueous methanol with formic acid. Approximately weighed amount 500 mg of #BRM 15898 samples were extracted by sonication method using different solvent composition [1, MeOH; 2, 1 % FA in MeOH; 3, 2 % FA in MeOH; 4, 80 % MeOH (1 % FA); 5: 80 % MeOH (2 % FA)]. The peak areas of all the reference compounds are compared among the five different extraction methods. Both acidified methanol (2 % FA) and 80 % MeOH (2 % FA) showed similar extraction efficiency.

Method validation

In present study, seven reference standards namely ajmaline (**1**), yohimbine (**2**), corynanthine (**3**), ajmalicine (**4**), serpentine (**5**), serpentinine (**6**), and reserpine (**7**) were quantified by using developed UHPLC-PDA method. The method was validated, and the results are given in Tables 1 and 2. The linearity was evaluated by analyzing the standard solutions of seven alkaloids at seven different concentration levels (1, 2, 5, 10, 20, 50, 100 μg/mL). According to the results, the linearity of the analytical response across the studied range is found to be good, with correlation coefficients (r^2) greater than 0.999. For all the seven reference standards, the LOD and LOQ were found to be in the ranges 0.1–0.5 and 0.5–1.0 μg/mL, respectively. System suitability test (RSD≤3.73 %) suggested the method was reproducible (Table 1). The intra- and inter-day RSD and % recovery of seven reference standards were found to be within the acceptable limits. Precision and accuracy results of the method were given in Table 2. For all seven reference standards proved to be stable at room temperature for 24 h and refrigeration temperature (4 °C) for 72 h. Robustness test was carried out by changing small changes in mobile phase composition, gradient program, flow rate, and column temperature. Robustness test has no significant change on efficiency of the method.

Analysis of plant samples and commercial products

The application of this method was determined by analyzing commercial products. One botanical reference material (#BRM 15898), one authenticated sample (#AU 7535), four commercial samples of *R. serpentina* (#CS 1623, #CS 1750, #CS 15949, #CS 16968), and one commercial sample of *R. vomitoria* (#CS 1631) were analyzed using this method. Botanical reference material (#BRM 15898) was used to develop fingerprint profile of *R. serpentina*, and this method validated by analyzing authenticated sample. One sample of *R. vomitoria* Afzel. (#CS 1631) purchased commercially was analyzed and found to have similar chemical constituents with that of authenticated *R. vomitoria* in context with the reported literature [10, 33]. The UV chromatograms at 272 nm of plant samples of *R. serpentina* (#BRM 15898, #AU 7535) and *R. vomitoria* (#CS 1631) showed a distinct peaks which can be used to differentiate these two species (Fig. 3). Further, this study was extended to UHPLC-QToF-MS in order to confirm the identity of the unknown peaks in methanolic extracts of plant samples which can also be used to differentiate these two species.

The compounds (**1**–**7**) in samples were identified by comparing retention time and UV spectrum of individual compounds in standard mixture. The UHPLC-UV chromatograms at 272 nm for plant samples and commercial products were

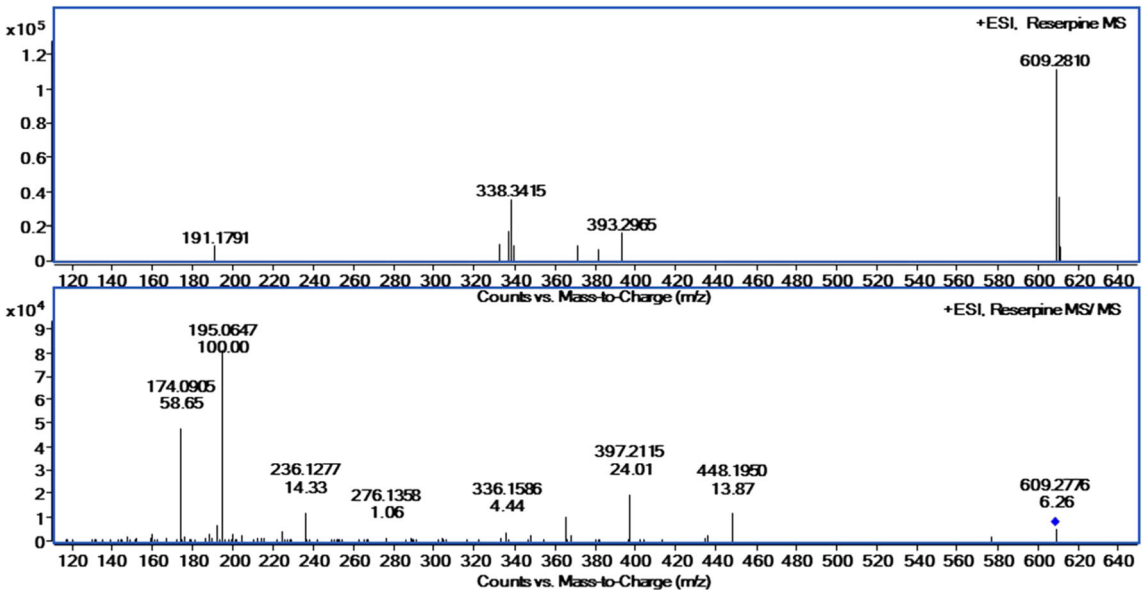

Fig. 2 Mass spectra of reserpine

shown in Fig. 3. The quantitative results for compounds (**1**–**7**) from the various plant samples are given in Table 3.

Concentrations are expressed as mg/g (*w/w*) for dry plant material. According to WHO monograph, *R. serpentina* contains not less than 1 % total alkaloids and a minimum of 0.1 % alkaloids of the reserpine-rescinnamine group [1]. Total alkaloidal content (**1**–**7**) was found to be in the range 4.9–12.2 mg/g of dry plant material. Botanical Reference Material (#BRM 15898) and authenticated sample (#AU 7535) showed all the seven reference compounds, and interestingly, the content of reserpine (**7**) one of most pharmacological active compound for treatment of hypertension was found lowest (0.39 mg/g) among the tested samples, and the content of serpentine (**5**) was found maximum 9.8 mg/g of dry plant material. In one commercial sample (#CS 1623), reserpine (**7**) was found maximum 1.3 mg/g of dry plant material. One commercial sample (#CS 1631) showed only three alkaloids (ajmaline, yohimbine, and reserpine). One commercial sample of *R. serpentina* (#CS 16968) showed only four alkaloids (ajmaline, yohimbine, ajmalicine, and reserpine). One authenticated sample (#AU 7535) and one commercial sample (#CS 1631) showed highest and lowest alkaloidal content (**1**–**7**) 12.2 and 4.9 mg/g of dry plant material, respectively. All seven plant samples analyzed showed reserpine and content was varied from 0.4 to 1.3 mg/g of dry plant material.

Fourteen dietary supplements were also analyzed by this method. Out of 14 products six claims to contain *R. serpentina* (#DS 15931–32, DS 15935, DS 15941–43), three claims to contain *R. serpentina* roots (#DS 15933, DS 15937 and DS 15945), one claim to contain *R. serpentina* root extract (#DS 15948), one claim to contain *R. serpentina* extract (#DS 15950), and three products claims to contain *R. vomitoria* root bark extract (#DS 15938, DS 15940, and DS 15947). In addition to these dietary supplements, seven pre-workout products claiming to contain *Rauwolfia* or *R. vomitoria* were also analyzed. All the seven pre-workout products also contain many other plant materials. The amount of alkaloids consumed daily was calculated based on the recommended daily usage provided on the label. The estimated maximum daily intake (mg/day) was calculated by multiplying the weight alkaloids

Table 1 Linearity, LOD, LOQ, and system suitability of 1–7 alkaloids

No.	Analyte	Retention time (min)	Linearity range (μg/mL)	Regression equation	r^2	LOD (μg/mL)	LOQ (μg/mL)	System suitability (% RSD)
1	Ajmaline	2.39	1-100	y=4389.7x-362.9	0.9993	0.5	1.0	0.29
2	Yohimbine	2.74	1-100	y=3446.1x-1144.5	0.9993	0.5	1.0	1.68
3	Corynanthine	3.04	1-100	y=4539x-3722.8	0.9994	0.5	1.0	2.25
4	Ajmalicine	5.36	1-100	y=7812.4x-6983.5	0.9994	0.5	1.0	1.45
5	Serpentine	5.74	1-100	y=18252x-20373	0.9993	0.1	0.5	0.57
6	Serpentinine	6.45	1-100	y=15263x-25342	0.9992	0.5	1.0	3.73
7	Reserpine	7.58	1-100	y=6375x-1399.8	0.9991	0.1	0.5	0.92

Table 2 Precision and accuracy for sample #BRM 15898

No.	Analyte	Intra-day (n=3)			Inter-day (n=9)	Recovery	
		Day 1	Day 2	Day 3		10 μg/mL	5 μg/mL
1	Ajmaline	0.33(3.75)	0.32(0.46)	0.34(1.91)	0.33(2.83)	93.71	91.82
2	Yohimbine	0.02(2.90)	0.02(0.77)	0.02(1.42)	0.02(2.87)	96.94	97.95
3	Corynanthine	0.02(3.39)	0.02(3.80)	0.02(2.40)	0.02(3.08)	95.83	97.89
4	Ajmalicine	0.05 (0.68)	0.05(1.68)	0.05(1.88)	0.05(3.42)	99.05	97.38
5	Serpentine	0.44(1.89)	0.44(0.84)	0.46(1.88)	0.45(3.04)	95.57	92.77
6	Serpentinine	0.17(2.75)	0.17(1.08)	0.18(2.18)	0.17(3.32)	85.90	94.79
7	Reserpine	0.05(3.45)	0.05(0.63)	0.05(1.75)	0.05(2.89)	95.25	93.84

Values are mean of triplicate (±RSD)

ND not detected

content (mg) by dilution factor by suggested maximum daily intake in capsules or tablets/ weight (mg) of content in capsules or tablets. For solid dosage forms, the suggested daily use varied from 2–10 capsules or 1–4 tablets; due to the difference in the composition of these samples, the daily intake also varied. The quantitative results for compounds (**1**–**7**) from the commercial product are given in Table 3. Total alkaloidal (**1**–**7**) content of 21 products varied from 0.0 to 4.5 mg/ recommended daily intake. Five products #DS 15928–29, # DS 15938, #DS 17019, and #DS 17028 does not contain any of the seven alkaloids. The content of reserpine varied from 0.002 to 0.08 mg/day in samples (#DS 15931–33, #DS 15935, #DS 15937, #DS 15940–43, #DS 15945). According to reported literature-recommended dose of reserpine is 0.1–0.25 mg/day [10]. Reserpine is present in all the plant samples analyzed irrespective of the species, but in 11 products, (#DS 15928–29, #DS 15938, #DS 15947–48, #DS 15950, #DS 17008, #DS 17016, #DS 17019, #DS 17027, and #DS 17028), reserpine was not detected (UV and MS). Ajmaline is major compound found in all plant samples analyzed, but only seven dietary supplements (#DS 15931, DS 15935, DS 15937, DS 15942–43, DS 15945, and DS 15948) showed ajmaline. Out of 21 products, yohimbine was not detected (UV and MS) in eight products (#DS 15928–29, DS 15937–38, DS 15947, DS 15950, DS 17019, and DS 17028) in which one product #DS 17028 claiming to contain 98 % yohimbine standardized extract. Ajmalicine, serpentine, and serpentinine are detected in few products but in very low amounts. One product (#DS 15947) contained only serpentine (0.9 mg/serving size). Seven products (# DS 15929, DS 15938, DS 17008, DS 17016, DS 17019, DS 17027–28) claim to contain 90 % rauwolscine, in which four products (#DS 15928, DS 15938, DS 17019, and DS 17028) where rauwolscine was not detected (MS). The only alkaloid detected in three (# DS 17008, DS 17016, and DS 17027) out of seven pre-work products was yohimbine (Table 3). The alkaloidal content is varied among the 21 commercial products.

Characterization of compounds by UHPLC-QToF-MS

The use of LC-mass spectrometry with electrospray ionization (ESI) method is described for the identification of seven compounds from roots samples of *Rauwolfia* species. This method involved the use of $[M+H]^+$ or $[M]^+$ ions of compounds **1**–**7** which were observed for reference compounds in positive ion mode. Further, the fragmentation patterns observed in the mass spectrum were useful in characterization of these compounds. Compounds from root samples and commercial products were identified by comparing their retention times and characteristic MS spectral data with those of reference compounds (Table 4). The base peak chromatograms of roots of *R. serpentina* and *R. vomitoria* extracts are shown in Fig. 4. Utilizing the high chromatographic resolution and separation capabilities of UHPLC with QToF-MS provides the structural characterization from accurate mass measurement for both MS and MS-MS experiments. These techniques offer a significant advantage for screening of target compounds from complex matrices.

In general, plants contain series of analogues having same skeleton with different functional groups; these compounds show similar MS fragmentation patterns. Therefore, investigating the fragmentation pattern of reference standards is practical accession to solve the identities of similar compounds in the same plant. The combination of both low (MS) and high (MS^2) collision offset voltage mode operations gave extra certainty to molecular mass determination. In addition, since the molecules are able to form adducts species in electrospray ionization source, their presence in low collision offset voltage spectra was very useful to carry out the unequivocal identification of $[M+H]^+$ ions and hence determining the molecular weight of unknown compounds. The study of high collision offset voltage spectra provided the fragmentation pathways of the different types of alkaloidal present in plant and plant products. *R. serpentina* is a rich source of alkaloids; hence, the abovementioned seven reference standards

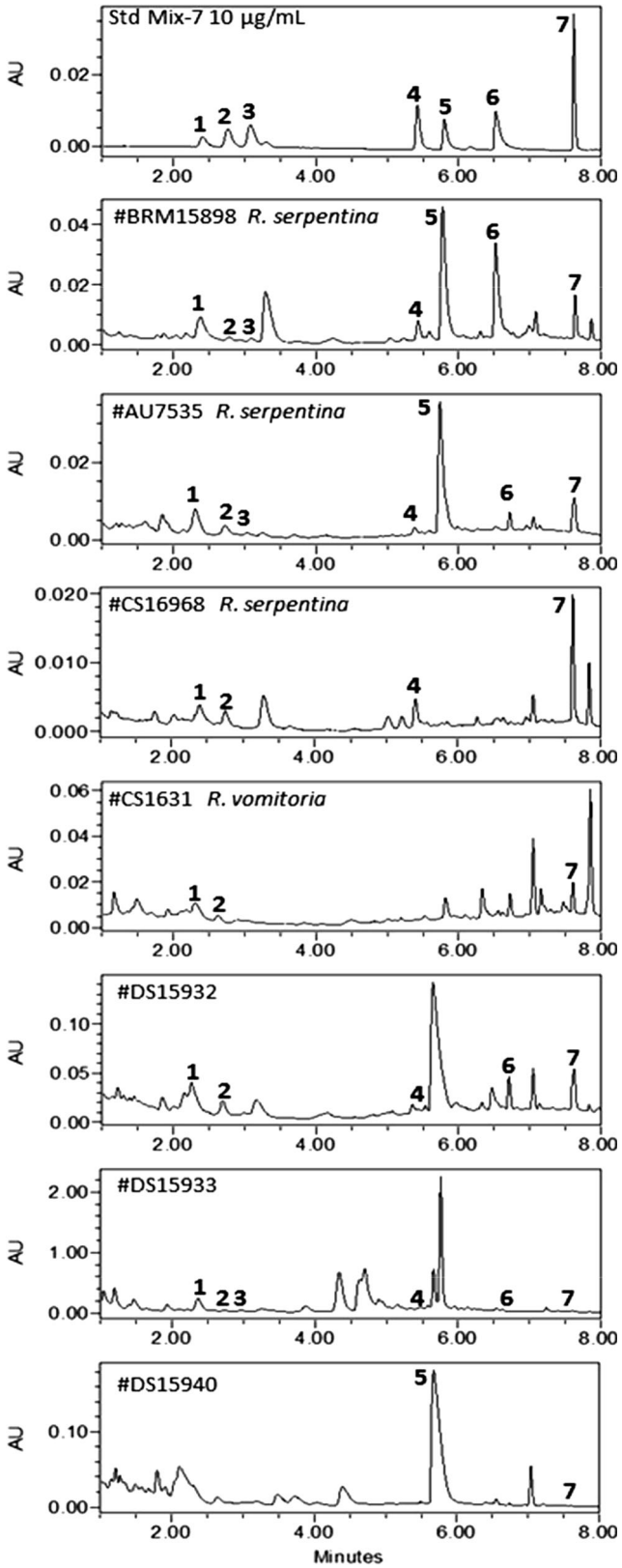

Fig. 3 UV chromatograms (272 nm) of Std Mix-7. Plant samples and dietary supplements: *1* ajmaline, *2* yohimbine, *3* corynanthine, *4* ajmalicine, *5* serpentine, *6* serpentinine, *7* reserpine

are taken for investigating the identity of similar compounds in the plant.

Reserpine is trimethoxybenzoic acid substituted alkaloid. CID spectra shows protonated ion $[M+H]^+$ at *m/z* 609.2807 and high collision offset voltage (40 V) spectra shows ions at *m/z* 174.0905, 195.0647, (100 %), 236.1277, 365.1839, 397.2115, and 448.1950. Neutral loss trimethoxybenzoic acid of from $[M+H]^+$ resulted in the formation of *m/z* 397.2115 $[C_{23}H_{29}N_2O_4^+]$. Loss of hydroxyl group from trimethoxybenzoic acid resulted in generation of most abundant peak at *m/z* 195.0647 $[C_{10}H_{11}O_4^+]$. Loss of CH_3OH from *m/z* 397.2115 yields an ion at *m/z* 365.1839 $[C_{22}H_{25}N_2O_3^+]$. Ring cleaving at C-2, 3, C-4, 5, and C-5,6 resulted in the formation of *m/z* 174.0905 $[C_{11}H_{12}NO^+]$ and *m/z* 448.1950 $[C_{23}H_{29}NO_8^+]$. Neutral loss of trimethoxybenzoic acid from *m/z* 448.1950 leads to generate *m/z* 236.1277 $[C_{13}H_{18}NO_3^+]$. The proposed fragmentation pattern of reserpine is shown in Fig. 5a.

Ajmaline is another type of indole alkaloid; low collision offset voltage mass spectra of ajmaline showed protonated ion at *m/z* 327.2067 $[M+H]^+$ and high collision offset voltage with 50 V showed ions at *m/z* 56.0500, 80.0496, 108.0805, 131.0726, 144.0802 (100 %), 158.0956, 182.0838, 194.1539, 210.1246, 220.1103, and 239.1521. *N*-Methyl indole derivative (*m/z* 158.0956) is formed by ring cleavage at C-2,3 and C-4,5, and further loss of methyl group from nitrogen atom yields the base peak at *m/z* 144.0802 $[C_{10}H_{10}N^+]$. Loss of C_8H_7NO from protonated ion gives *m/z* 194.1539 $[C_{12}H_{20}NO^+]$ and cleavage at C-2,3 and C-4,5 from *m/z* 194.1539 yields *m/z* 108.0808$[C_7H_{10}N^+]$ which further dissociates to form *m/z* 80.0496$[C_5H_6N^+]$ and *m/z* 56.0500$[C_3H_6N^+]$. The proposed fragmentation pattern of ajmaline is shown in Fig. 5b.

Yohimbine, MS spectra of yohimbine showed protonated ion at *m/z* 355.2016 $[M+H]^+$ and MS/MS showed ions at *m/z* 117.0691, 144.0805, 162.0901, 180.1005, 194.1168, 212.1277, 224.1276, and the most abundant ion was found at *m/z* 144.0805 (100 %) and ions at *m/z* 212.1277 and *m/z* 224.1276 are the other ions showed up with considerable abundance. Ring cleavage at C-2,3 and C-4,5 resulted in the formation of indole derivative with *m/z* 144.0805 $[C_{10}H_{10}N^+]$ and *m/z* 212.1277 $[C_{11}H_{18}NO_3^+]$. Similarly, ring cleavage at C-2,3 and C-5,6 resulted in formation of *m/z* 224.1276 $[C_{12}H_{18}NO_3^+]$. Other few peaks were observed in MS^2 spectra of yohimbine were clearly represented in Fig. 6a. These three peaks are characteristic peaks for the identifying the similar yohimbine type of compound like corynanthine.

Collision-induced dissociation (CID) spectra of ajmalicine at low collision offset voltage showed protonated ion at *m/z* 353.1826 and high collision offset voltage (30 V) showed ions at *m/z* 117.0684, 144.0799 (100 %), 178.0845, 210.1108, 220.1140, and 252.0094. The fragmentation behavior of ajmalicine is similar to that of yohimbine, except the presence

Table 3 Content mg/g, *w/w* of compounds (1–7) from plant samples and mg/recommended daily intake from dietary supplements claiming to *R. serpentina*, *R. vomitoria* and *Rauwolfia* extract; 1-ajmaline; 2-yohimbine; 3-corynanthine; 4-ajmalicine; 5-serpentine; 6-serpentinine; 7-reserpine

S. No	No.		1	2	3	4	5	6	7	Total[a]
Plant samples		Plant name	Alkaloidal content mg/g of dry plant material							
1	BRM 15898	*R. serpentina* roots	3.41	0.24	0.19	0.48	4.46	1.76	0.52	11.1
2	AU 7535	*R. serpentina* roots	0.59	0.36	0.14	0.11	9.83	0.23	0.39	11.7
3	CS 1623	*R. serpentina* roots	2.96	0.41	0.24	0.61	3.60	1.46	1.31	10.6
4	CS 1750	*R. serpentina* roots	2.00	0.68	0.12	0.17	6.79	1.36	0.96	12.1
5	CS 15949	*R. serpentina* roots	2.67	1.07	DUL	0.34	0.12	ND	0.45	4.65
6	CS 16968	*R. serpentina* roots	1.21	0.24	ND	ND	0.11	ND	DUL	1.57
7	CS 1631	*R. vomitoria* roots	3.87	0.36	ND	ND	ND	ND	0.48	4.71
Dietary supplements		Plants used in products	Alkaloidal content mg/recommended daily intake							
1	DS 15931	*R. serpentina*	0.04	0.02	0.002	ND	0.10	0.008	0.008	0.18
2	DS 15932	*R. serpentina*	ND	0.09	0.09	0.02	ND	0.05	0.08	0.33
3	DS 15935	*R. serpentina*	0.05	0.06	ND	0.006	0.005	0.005	0.03	0.16
4	DS 15941	*R. serpentina*	ND	0.03	0.009	ND	ND	ND	0.03	0.07
5	DS 15942	*R. serpentina*	0.12	0.04	ND	ND	0.13	0.009	0.04	0.34
6	DS 15943	*R. serpentina*	0.14	0.07	ND	0.01	0.06	0.02	0.04	0.34
7	DS 15933	*R. serpentina* roots	ND	0.04	0.03	ND	ND	ND	0.03	0.10
8	DS 15937	*R. Serpentina roots*	0.03	ND	ND	ND	ND	ND	0.01	0.04
9	DS 15945	*R. serpentina* roots	0.10	0.04	ND	ND	ND	ND	0.03	0.17
10	DS 15948	*R. serpentina* root extract	0.03	0.005	0.01	ND	0.02	0.006	ND	0.07
11	DS 15950	*R. serpentina* extract	ND	ND	ND	ND	0.005	0.004	ND	0.01
12	DS 15938	*R. vomitoria* root bark extract	ND	ND	ND	ND	ND	ND	ND	0
13	DS 15940	*R. vomitoria* root bark extract	ND	0.04	ND	ND	0.58	0.01	0.002	0.63
14	DS 15947	*R. vomitoria* root bark extract	ND	ND	ND	ND	0.93	ND	ND	0.93
Pre-workout products		Plants used in products	Alkaloidal content mg/recommended daily intake							
15	DS 17019	*R. vomitoria* root bark extract	ND	ND	ND	ND	ND	ND	ND	0
16	DS 17027	*R. vomitoria* root bark extract	ND	4.47	ND	ND	ND	ND	ND	4.47
17	DS 17028	*R. vomitoria* root bark extract	ND	ND	ND	ND	ND	ND	ND	0
18	DS 15929	*R. vomitoria* root extract	ND	ND	ND	ND	ND	ND	ND	0
19	DS 17016	*R. vomitoria* root extract	ND	1.24	ND	ND	ND	ND	ND	1.24
20	DS 17008	*R. vomitoria*	ND	1.41	ND	ND	ND	ND	ND	1.41
21	DS 15928	*Rauwolfia* extract	ND	ND	ND	ND	ND	ND	ND	0

[a] Total: sum of seven alkaloids

ND not detected, *DUL* detection under the limits of quantification

of double bond (C-16, 17), oxygen atom in ring D, and methyl group at C-19 position. The proposed fragmentation pattern of ajmalicine is depicted in Fig. 6b.

Serpentine is a quaternary alkaloid, and CID spectra shows molecular ion at *m/z* 349.1514 [M^+] and at high collision offset voltage spectra shows ions at *m/z* 317.1256, 289.1311, 277.0944, 263.0796, 235.0849 (100 %), 207.0900, and 182.0818. Loss of CH_3OH from molecular ion resulted in generation of *m/z* 317.1256 [$C_{20}H_{17}N_2O_2^+$] which further loss of CO yields ion at *m/z* 289.1311 [$C_{19}H_{17}N_2O^+$]. Cleavage of ring D at C-4, 21, C-15 leads to formation of *m/z* 182.0818 [$C_{12}H_{10}N_2^{+\cdot}$] with radical nitrogen. Simultaneous cleavage of ring D-E at C-4, 21, C-15, 20; C-18, 19 and loss CH_3OH from molecular ion resulted in lactone formation with *m/z* 263.0796 [$C_{16}H_{11}N_2O_2^+$]. Consecutive loss of two CO molecules from *m/z* 263.0815 resulted in the formation of most abundant ion *m/z* 235.0849 [$C_{15}H_{11}N_2O^+$] and *m/z* 207.0900 [$C_{14}H_{11}N_2^+$]. Loss of CH_2 and cleavage of ring E at C-19, 20, and C-16, 17 resulted in formation of *m/z* 277.0944 [$C_{17}H_{13}N_2O_2^+$]. Proposed mass fragmentation pattern is shown Fig. 7a.

Serpentinine is a quaternary alkaloid with dimeric structure; CID spectra shows molecular ion [M^+] at *m/z* 685.3384 and collision offset voltage (60 V) spectra shows ions at *m/z* 144.0799, 251.1530, 343.1428, 375.1691(100 %), 435.1914, 621.2822, and 653.3079. Consecutive loss of two CH_3OH molecules from protonated ion leads to generation of ions at

Table 4 Tentatively identified compounds from roots of *R. serpentina* using LC-QToF-MS

No.	Compound	t_R (min)	Molecular formula	Accurate mass *m/z* $[M+H]^+$	Exact mass *m/z* $[M+H]^+$	PPM error	Fragment ions	#BRM 15898	#AU 7535	#CS 1623	#CS 1750	#CS 15949	#CS 16968	#CS 1631
1	Peraksine	1.94	$C_{19}H_{22}N_2O_2$	311.1752	311.1754	0.73	160.0754, **138.0906,** 120.0805	+	+	+	+	+	+	+
2	Nortetraphyllicine	2.89	$C_{19}H_{22}N_2O$	295.1807	295.1805	−0.85	277.1690, **144.0801,** 130.0646	ND	ND	ND	ND	ND	ND	+
3	Norajmaline	2.99	$C_{19}H_{24}N_2O_2$	313.1916	313.1911	−1.69	158.0925, **144.0795,** 138.0902	+	+	+	+	+	+	+
4	Sarpagine	3.79	$C_{19}H_{22}N_2O_2$	311.1760	311.1754	−1.81	**150.0906**, 144.0804**,** 122.0959	+	+	+	+	+	+	+
5	Tombozine	4.30	$C_{19}H_{22}N_2O$	295.1811	295.1805	−2.21	144.0801**,** **138.0910,** 120.0804	+	+	+	+	+	+	ND
6	Isoajmaline	4.63	$C_{20}H_{26}N_2O_2$	327.2079	327.2067	−3.54	212.1083, 158.0957, **144.0806**	+	+	+	+	+	+	+
7	Rauwolscine	4.82	$C_{21}H_{26}N_2O_3$	355.2024	355.2016	−2.21	224.1276, 212.1277, **144.0804**	+	+	+	+	+	+	+
8	**Ajmaline**	5.12	$C_{20}H_{26}N_2O_2$	327.2078	327.2067	−3.31	194.0956, 158.0957, **144.0804**	+	+	+	+	+	+	+
9	Vinorine	6.03	$C_{20}H_{18}N_2O_3$	335.1395	335.1390	−1.31	317.1270**,** **263.0808,** 144.0805	+	+	+	+	+	ND	+
10	**Yohimbine**	6.19	$C_{21}H_{26}N_2O_3$	355.2024	355.2016	−2.21	224.1266, 212.1268, **144.0804**	+	+	+	+	+	+	+
11	Hydroxyyohimbine	6.22	$C_{21}H_{27}N_2O_4$	371.1969	371.1965	−1.08	355.2007, 212.1271, **144.0809**	+	+	+	+	+	+	+
12	**Corynanthine**	6.68	$C_{21}H_{26}N_2O_3$	355.2002	355.2016	4.07	224.1261, 212.1268, **144.0784**	+	+	+	+	+	+	+
13	Geissoschizine derivative	7.00	$C_{27}H_{30}N_2O_8$	511.2084	511.2075	−1.70	**349.1548,** 212.1274, 144.0802	+	+	+	+	+	+	+
14	Strictosidine	7.92	$C_{27}H_{34}N_2O_9$	531.2346	531.2337	−1.61	224.1271, 212.1272, **144.0800**	+	+	+	+	+	ND	+
15	Ajmalicine isomer	8.43	$C_{21}H_{24}N_2O_3$	353.1854	353.1860	1.55	222.1114, 210.1116, **144.0808**	+	+	+	+	+	+	ND
16	**Ajmalicine**	9.81	$C_{21}H_{24}N_2O_3$	353.1875	353.1860	−4..25	222.1125, 210.1110, **144.0808**	+	+	+	+	+	+	+
17	**Serpentine**[a]	10.46	$C_{21}H_{21}N_2O_3$	349.1547	349.1547	−1.67	263.0796, **235.0866,** 207.0900	+	+	+	+	+	+	ND
18	Acetylgeissoschizol	10.81	$C_{21}H_{26}N_2O_2$	339.2072	339.2067	−1.52	**158.0960,** 136.0750, 108.0804	ND	ND	ND	ND	ND	ND	+
19	Vomilenine	13.24	$C_{22}H_{23}N_2O_4$	351.2064	351.2067	0.78	182.0943, 158.0952**,** **144.0800**	ND	ND	ND	ND	ND	ND	+
20	Serpenticine	14.07	$C_{22}H_{23}N_2O_4$	379.1650	379.1652	0.69	347.1370, **293.0910,** 144.0793	+	+	+	+	+	+	ND
21	Unknown	15.23	$C_{42}H_{44}N_2O_5$	685.3378	685.3384	0.87	433.1775, 375.1465, **144.0799**	ND	ND	ND	ND	ND	ND	+
22	**Serpentinine**[a]	15.83	$C_{42}H_{45}N_2O_5$	685.3372	685.3384	1.78	435.1894, **375.1691,** 144.0807	+	+	+	+	+	ND	ND
23	Raunescine	17.03	$C_{32}H_{38}N_2O_9$	595.2629	595.2650	3.46	383.1954, **195.0643,** 174.0897	ND	ND	ND	ND	ND	ND	+
24	**Reserpine**	22.59	$C_{33}H_{40}N_2O_9$	609.2795	609.2807	1.86	397.2115, **195.0670,** 174.0905	+	+	+	+	+	+	+
25	Rauvomitine	22.80	$C_{30}H_{34}N_2O_5$	503.2558	503.2540	−3.40	291.1847**,** **195.0647,** 158.0954	ND	ND	ND	ND	ND	ND	+
26	Rescinnamine	22.89	$C_{35}H_{42}N_2O_9$	635.2989	635.2963	−4.03	607.2670, 397.2110**,** **221.0804**	+	+	+	+	+	+	+

Geissoschizine derivative-3,4,5,6-Tetradehydro geissoschizine-17-*O*-β-D-glucopyranoside; compounds marked in bold are reference compounds; values with bold and underlined are 100 % abundance ion

ND not detected

[a] M^+ ion

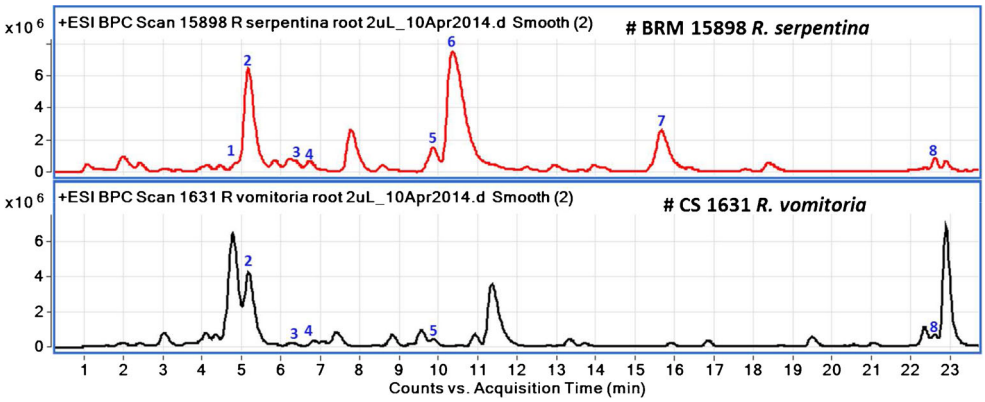

Fig. 4 Base peak chromatograms of roots of *R. serpentina* and *R. vomitoria*

m/z 653.3079 [$C_{41}H_{41}N_4O_4^+$] and *m/z* 621.2822 [$C_{40}H_{37}N_4O_3^+$] respectively. Serpentinine is a combination of quaternary and tertiary alkaloids; with high collision energy, the molecule is dissociated into two ions *m/z* 435.1914 [$C_{25}H_{27}N_2O_5^+$] and *m/z* 251.1543 [$C_{17}H_{19}N_2^+$]. Simultaneous loss of CH_3OH and CO from *m/z* 435.1914 leads to formation of most abundant ions at *m/z* 375.1691 [$C_{23}H_{23}N_2O_3^+$], further loss of CH_3OH leads to the generation of *m/z* 343.1441 [$C_{22}H_{19}N_2O_2^+$]. Ion at *m/z* 144.0799 [$C_{10}H_{10}N^+$] can be formed from one of the indole rings present in molecule. The proposed fragmentation pattern of serpentinine is shown in Fig. 7b.

With the detailed mass fragmentation behavior of the reference standards and reported literature, the identification of similar compounds in the plant samples was investigated. Tentatively identified compounds from *R. serpentina* with retention time, molecular formula, accurate mass, extact mass, ppm error, major fragment ions are listed in Table 4.

The application of the LC-ESI-MS/MS technique in the current study provided useful information to characterize seven references, 16 known, and one unknown compound from *R. serpentina* and *R. vomitoria* through the use of authenticated plant materials. Identification of specific markers for *R. serpentina* and *R. vomitoria* was done by analyzing samples #BRM 15898, #AU 7535, and #CS 1631. Two reference compounds, serpentine and serpentinine, are known to be markers for *R. serpentina*, and compounds tombizine (t_R=4.30), ajmalicine isomer (t_R=8.43), unknown (t_R=16.27) were found to be additional markers to identify *R. serpentina*. Compounds nortetraphylline (t_R=2.89), acetylgeissoschizol (t_R=10.81), vomilenine (t_R=13.24), raunescine (t_R=17.03), rauvomitine (t_R=22.80), and unknown (t_R=15.23) were found to be markers to identify *R. vomitoria*. Five compounds, ajmaline, yohimbine, corynanthine, ajmalicine, reserpine, and 12 other compounds were found in both species with content of individual alkaloids may vary. For example, isoajmaline and reserpine were found to be high amount in *R. vomitoria* than *R. serpentina* whereas vinorine and norajmaline were found to be high amount in *R. serpentina* than *R. vomitoria.* Two peaks at t_R, 15.23 and 15.83 min with same *m/z* 685.3372 and mass fragmentation pattern were used as one of the distinct markers for two species. The latter compound was confirmed as

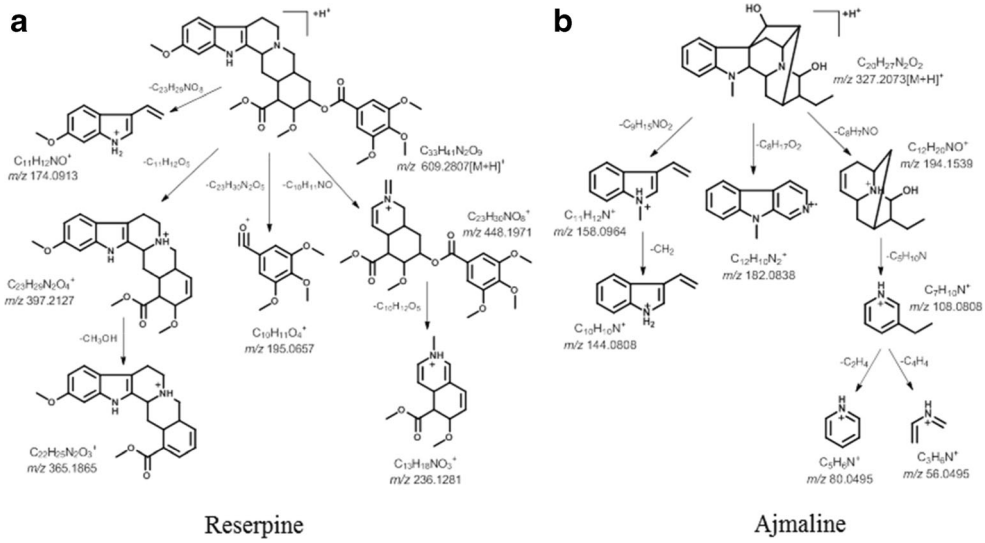

Fig. 5 Proposed mass fragmentation pattern of **a**) reserpine and **b**) ajmaline

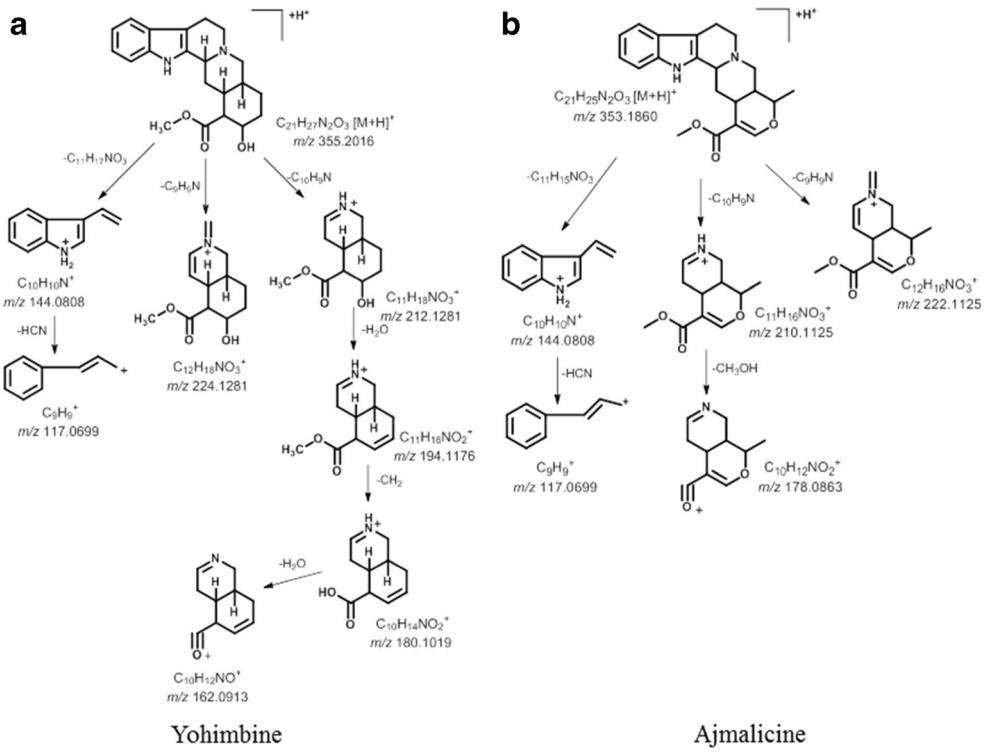

Fig. 6 Proposed mass fragmentation pattern of **a**) yohimbine and **b**) ajmalicine

serpentinine, and the former was not able to identify but the high content of this compound is present in sample #CS 1631. Some compounds showed same *m/z*, but different fragment ion (base peak), e.g., peraksine and sarpagine, showed *m/z* 311.1752 whereas the base peak was showed at *m/z* 138.0906 and 144.0804, respectively. Similarly, serpentine and alstonine were identified with fragment ion at *m/z* 235.0866 and 263.0810, respectively, and they are chromatographically separable. Most of the compounds showed *m/z* 144.0804 as base peak which confirms the identity of substituted indole ring. The compounds **23**–**26** listed in Table 4 were identified with major fragment ion at *m/z* 195.0647 which corresponds to trimethoxybenzoic acid moiety in reserpine.

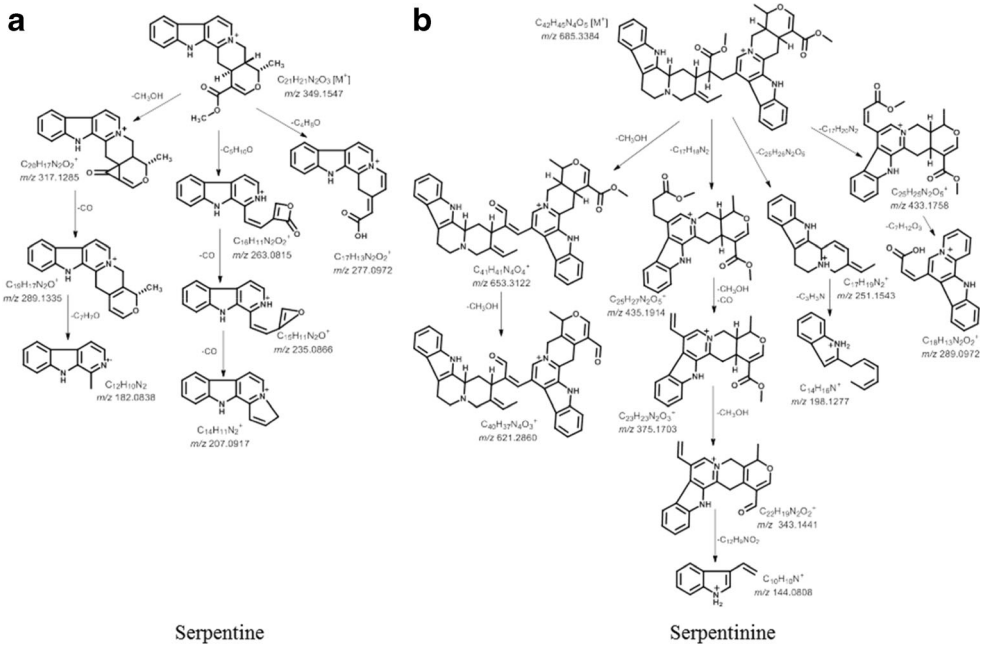

Fig. 7 Proposed mass fragmentation pattern of **a**) serpentine and **b**) serpentinine

Conclusions

A simple and efficient UHPLC-PDA method was developed for the quantification of seven alkaloids from roots of *Rauwolfia* species (*R. serpentina* and *R. vomitoria*) and commercial products. The method was validated in terms of linearity, LOD, LOQ, intra- and inter-day precision, recovery, system suitability, stability, and robustness. Seven *Rauwolfia* root samples, 14 dietary supplements, and seven pre-workout products were analyzed. In the root samples, the amount of reserpine and total alkaloidal (**1**–**7**) content was found to be in the range 0.4–1.3 and 1.6–12.1 mg/g of dry plant material, respectively. Whereas in commercial products, reserpine and total alkaloidal (**1**–**7**) content of dietary supplements were found to be in the range of 0.0–0.1 and 0.0–4.5 mg/day, respectively. The quantitative data shows the *Rauwolfia* commercial products showed wide variations in the content of alkaloids. UHPLC-QToF-MS/MS technique was applied for the characterization of alkaloids from *Rauwolfia* species. The characteristic fragmentation patterns observed in QToF-MS/MS spectra allow the identification and characterization of the compounds. The fragmentation behavior of all seven reference compounds was discussed in detail. Reserpine showed major fragment ion at *m/z* 195.1 with 100 % abundance. Ajmaline, yohimbine, corynanthine, and ajmalicine showed *m/z* 144.0 as major fragment ion. The developed analytical methods including MS-MS characterization would be valuable not only for herbal identification but also for quality control of commercial products containing *R. serpentina* or *R. vomitoria*.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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