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Rapid fingerprinting of Rauwolfia species using direct analysis in real time mass spectrometry combined with principal component analysis for their discrimination

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Abstract: Medicinal plants of the genus *Rauwolfia* (Apocynaceae) are extensively used as folk medicines worldwide. Its antihypertensive activity is well known due to the presence of monoterpene indole alkaloids (MIAs). The therapeutic potential of the herbal medicines are affected due to variation of bioactive phytoconstituents. Therefore, a rapid and validated method was developed for fingerprinting of roots and leaves of six *Rauwolfia* species by direct analysis in real time mass spectrometry (DART-MS). Seventeen bioactive MIAs were tentatively identified on the basis of their exact mass measurement from the intact plant parts. Further, principal component analysis (PCA) was used to analyze the DART-MS data of six *Rauwolfia* species to identify the chemical markers. Thirteen and twenty three chemical markers were identified from roots and leaves which were able to discriminate among six *Rauwolfia* species. This method was also cross-validated for the rapid identification, authentication and quality control of *Rauwolfia* species.

1. Introduction

Traditional medicines have become increasingly popular worldwide because of its time tested therapeutic potential with minimum side effects.[1-2] However, variation in the bioactive phytoconstituents of herbal drugs significantly affects their therapeutic efficacy and undermines the practice of herbal medicines itself.[3] Potent medicinal plants of *Rauwolfia* species

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(Apocynaceae) are taxonomically and morphologically similar and distributed worldwide.[4-6] It is widely used as traditional medicine due to presence of bioactive monoterpene indole alkaloids (MIAs).[7-9] The *Rauwolfia* species such as *R. hookeri, R. micrantha, R. serpentina, R. tetraphylla, R. verticillata, R. vomitoria* and *R. serpentina* has been used in Ayurveda and Indian system of traditional medicine for the treatment of high blood pressure, hypertension and various central nervous system related psychotic diseases.[10-13] The roots and leaves are used in intestinal disorders, particularly diarrhoea and dysentery and also as an antihelmentic to treat cholera, colic and fever.[13-16] Identification and authentication of *Rauwolfia* species is difficult due to more morphological similarity. Various analytical methods such as nuclear magnetic resonance (NMR),[17-19] high performance thin layer chromatography (HPTLC), [4] high performance liquid chromatography (HPLC), [20, 21] gas chromatography mass spectrometry (GC/MS),[21] and liquid chromatography-mass spectrometry (LC/MS), [6, 22] are used to identify and quantify bioactive compounds in *Rauwolfia* species. These methods are sensitive toward physical state of sample (solid, liquid, gas), concentration, and require time-consuming sample preparation steps. To overcome these analytical difficulties, there are various ambient mass spectrometric techniques such as direct analysis in real time mass spectrometry (DART-MS), desorption electrospray ionization (DESI), extractive electrospray ionization (EESI), paper spray, leaf spray, wooden-tip ESI can be used.[23-27] DESI and EESI produces multiple charged ions and adducts while paper spray (PS), leaf spray (LS) wooden-tip ESI require sample pre-treatment with wipe solvent.[25-28] However DART-MS ionizes samples directly without sample pre-treatment and gives singly charged ions hence selected for analysis.[29]

DART-MS is useful tool for analysis of small molecules by reaction of electronic or vibrionic excited-state species (metastable helium) with the analytes.[30] DART-MS has been successfully applied for metabolic profiling of pharmaceuticals counterfeit drugs, bacterial fatty acid methyl esters, flavours and fragrances, pesticides, and adulteration. [31-43] The DART-MS fingerprinting of medicinal plants in combination with multivariate analysis techniques such as principal component analysis has revealed its potential for analysis of medicinal plant metabolites and their discrimination.[44-48] In the present study, we have developed the chemical fingerprinting of *Rauwolfia* species for identification and distribution of MIAs. Furthermore, DART-MS data obtained were analyzed by principle component analysis (PCA) to identify the chemical markers which were able to discriminate among the six *Rauwolfia* species.

2. Materials and methods

2.1. Plant materials

The roots and leaves of *R. hookeri, R. micrantha, R. serpentina, R. tetraphylla, R. verticillata* and *R.*

vomitoria were collected in September 2012 from plants grown under similar conditions in Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) campus (N: 8^0 45', E: 77^0 10', Altitude: 70-160m), Kerala (India). Voucher specimens (*R. hookeri*- 66449*, R. micrantha*- 66450*, R. serpentina*- 66451, *R. tetraphylla*- 66452, *R. verticillata*- 66453, *R. vomitoria*- 66454) are deposited in the Herbarium of JNTBGRI. The roots and leaves were washed with tap water and dried at room temperature prior to analysis.

2.2. DART-MS Analysis

The mass spectrometer used was a JMS-100 TLC (AccuTof) atmospheric pressure ionization time-of-flight mass spectrometer (Jeol, Tokyo, Japan) fitted with a DART ion source. The mass spectrometer was operated in positive-ion mode with a resolving power of 6000 (full-width at half-maximum). The orifice 1 potential was set to 28 V, resulting in minimal fragmentation. The ring lens and orifice 2 potentials were set to 13 and 5 V, respectively. Orifice 1 was set to a temperature of 100°C. The RF ion guide potential was 300 V. The DART ion source was operated with helium gas flowing at approximately 4.0 L/min. The gas temperature was optimized and 300°C was found suitable for ionization. The potential on the discharge needle electrode of the DART source was set to 3000 V; electrode 1 was 100 V and the grid was at 250 V. Data acquisition was from *m/z* 10 to 1000. Exact mass calibration was accomplished by including a mass spectrum of neat polyethylene (PEG) glycol (1:1 mixture PEG 200 and PEG 600) in the data file. The elemental composition was determined on selected peaks using the Mass Center software.

2.3 Principal component analysis (PCA)

PCA was performed using STATISTICA software, Windows version 7.0 (Stat Soft, Inc., USA). Three samples of each part (roots and leaves) were recorded in 15 repeats to check the repeatability and reproducibility of spectra. All ions having ≥5% relative intensity were selected for PCA. 10 repeats used to build the PCA model for discrimination and the remaining 5 repeats were used for cross-validation and testing of the PCA model.

3. Results and Discussion

3. 1. DART-MS analysis of six *Rauwolfia* species

Comparative DART-MS fingerprints spectra of the roots and leaves of *R. hookeri, R. micrantha, R. serpentina, R. tetraphylla, R. verticillata* and *R. vomitoria* are shown in Fig. 1 and 2. Seventeen MIAs were tentatively identified based on their exact mass, molecular formula and literature reports [7, 8, 20, 21] as shown in Table 1. The identified compound were confirmed and supported by their MS/MS fragmentation pattern (Fig. S3-S5). These components were directly ionized from roots and leaves during analysis and appeared as protonated molecular ions $[M+H]^+$ in the resulting spectra. The peaks at *m/z* 327 ($C_{20}H_{27}N_2O_2$), 341 ($C_{21}H_{28}N_2O_2$) and 351 ($C_{21}H_{23}N_2O_3$) could be due to ajmaline,

sandwicolidine and vomilenine, respectively which were detected in relatively high abundance in roots. Ajmaline was detected in roots of *R. verticillata* and *R. serpentine* in higher abundance while sandwicolidine was found abundant in roots of *R. tetraphylla* and *R. vomitoria.* Similarly, vomilenine was identified in high abundance in roots of *R. hookeri* and *R. micrantha.* Peak at *m/z* 355 ($C_{23}H_{27}N_2O_3$) was identified as yohimbine and found relatively high in *R. serpentina* followed by *R. vomitoria* and *R. tetraphylla* roots. The peak at *m/z* 413 ($C_{19}H_{25}N_2O_2$) was identified as reserpiline in roots of *R. verticillata, R. micrantha* and *R. hookeri* in relatively less intensity. Reserpiline (*m/z* 413) was abundant in *R. vomitoria* followed by *R. micrantha R. tetraphylla* and *R. hookeri* while yohimbine (*m/z* 355) was high in intensity in *R. tetraphylla* followed by *R. hookeri*, *R. vomitoria* and *R. micrantha* leaves. *R. serpentina* and *R. verticillata* (roots) showed more peaks than others.

The DART-MS spectra revealed the variation in the distribution of some of the most common MIAs in the roots and leaves of six species in the terms of percent ionization as shown in Fig. 3. It was obtained as the ratio of the expression of the peak to the sum of all the expressions within the spectra ranging from *m/z* 100-700. All the ions with relative intensity above 5% were taken and compared on the basis of (%) ionization. Fifteen repeats were carried out for each sample and averaged result was utilized for this analysis. Since, the DART-MS technique involves desorption ionization and is carried out in native conditions. Hence, obtained results may be used for the relative quantification amongst six *Rauwolfia* species. Results indicated significant variations of bioactive compounds among the roots and leaves of six *Rauwolfia* species (Fig. 3A and 3B). Sarpagine (*m/z* 311) was present in relatively abundance in leaf of *R. serpentina* followed by *R. verticillata*. Ajmaline (*m/z* 327) was higher in *R. verticillata* followed by *R. serpentina, R. vomitoria* and *R. tetraphylla* roots while it was detected only in leaf of *R. micrantha.* Ajmalicine (*m/z* 353) was found only in *R. vomitoria* root and in case of leaf it was higher in *R. tetraphylla* followed by *R. verticillata.* Yohimbine (*m/z* 355) was found higher in root of *R. serpentina* followed by *R. vomitoria* and *R. tetraphylla* and leaf showed higher content in *R. tetraphylla* followed by *R. hookeri, R. vomitoria and R. micrantha.* Reserpiline (*m/z* 413) was detected maximum in *R. hookeri* followed by *R. micrantha* and *R. verticillata* roots. Reserpiline was found higher in *R. vomitoria* followed by *R. hookeri* and *R. verticillata* in leaf (Fig.3).

This observation will clearly help to select the most suitable plant/parts for medicinal purposes on the basis of relative abundance of the bioactive compounds. The characteristic chemical fingerprints of raw *Rauwolfia* species obtained by DART-MS analysis proved the versatility of this technique and these results may also be used for the quality control of these medicinal plants.

3. 2. **Identification of chemical markers using principal component analysis**

DART-MS data combined with data reduction technique such as principal component analysis (PCA) serves as an efficient and powerful tool to identify the chemical markers for discrimination. The DART-MS chemical fingerprint of *R. hookeri, R. micrantha, R. serpentina, R. tetraphylla, R. Verticillata*, *R. vomitoria* roots and leaves were analyzed by principal component analysis (PCA) to identify the chemical markers for discrimination amongst the *Rauwolfia* species.

19 and 37 peaks were taken for PCA from roots and leaves in the range of *m/z* 100-700. The first two principal components PC1 and PC2 hold 37.05% and 25.53% respectively of the total variability in roots (Fig. S1A). Similarly, principal components PC1 and PC2 hold 38.08% and 27.18% respectively of the total variability in leaves (Fig. S1B). Thus, the PCs were able to explain 62.58% (roots) and 61.26% (leaves) of the total variability on the basis of total peaks. To obtain the best expression some peaks were dropped which having low scores to get the best possible results. Finally, first two principal components PC1 and PC2 hold 50.64% and 30.75% respectively of the total variability on the basis of 13 peaks (*m/z* 296, 326, 327, 341, 343, 351, 355, 371, 411, 412, 413, 429, and 653) from roots (Fig. 4A). Similarly 23 peaks (*m/z* 183, 248, 274, 275, 276, 277, 279, 293, 295, 308, 310, 311, 312, 351, 391, 395, 409, 411, 412, 413, 429, 439 and 457) from leaves showed two principal components PC1 and PC2 hold 56.02% and 31.93% respectively of the total variability (Fig. 4B). Hence, 13 and 23 peaks from roots and leaves showed 81.39 and 87.95 variance respectively therefore it is best possible results. Loadings plot of *Rauwolfia* species; the quadrants marked A–D correspond to those shown in Fig.S2 A and B. Loadings can range from 1 to -1 and are usually described as the cosine of the angle between the variable axis and the principal component axis. Each data point represents *m/z* of peaks which give rise to variance in six *Rauwolfia* species are found in all quadrants. All 13 and 23 marker peaks are responsible for variance distribution. The *m/z* 296 (50.87%) gave higher contribution for discrimination followed by *m/z* 326 (30.23%) for roots. Similarly *m/z* 183 and 248 gave contribution at 57.08% and 32.17% respectively for leaves. *R. verticillata* is distinct from all other species. R. *tetraphylla, R. vomitoria* and *R. hookeri* are much closer to each others. Similarly, *R. tetraphylla R. vomitoria* and *R. serpentina* are approximately similar falling to the same quadrant. *R. serpentina* root showed the similarity with the root of *R. tetraphylla* and *R. vomitoria* whereas its leaf is much more apart from all six species. *R. micrantha* roots are showing similarity with *R. hookeri*. The proposed PCA method was cross-validated and is shown in Fig. 4 and S1 (marked red). It is evident from this study that PCA effectively served the purpose. The six *Rauwolfia* species could be differentiated by this validated method.

Conclusions

Rapid and simple DART-MS method was developed for the analysis of six *Rauwolfia* species. Seventeen MIAs were tentatively identified and their variations in leaf and root were studied. Results showed significant qualitative variations amongst *Rauwolfia* species. These findings will help in selection of the best suitable plant/part, according to the requirement and may be used for the authentication and quality control purposes. The marker peaks were identified successfully by PCA which are able to discriminate *R. hookeri, R. micrantha, R. serpentina, R. tetraphylla, R. verticillata* and *R. vomitoria*. It is evident from this study that PCA effectively served the purpose and all the six *Rauwolfia* species could be differentiated by this validated method.

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References

1 V. P. Kamboj Curr. Sci., 2000, **78**, 35-51

2 M. Philipp, R. Kohnen, K. Hiller. J. Clin sychaitry, 1999, **319**, 1534–1538

3 WHO fact, traditional medicine sheet no.134.

4 P. A. Cox, M. J. Balick, *Sci. Am.,* 1994, **270**, 60-65.

5 D. J. Mabberley, *Mabberley's plant-book: a portable dictionary of plants, theirclassifications, and uses*, Cambridge University Press, **2008**.

6 S. Bindu, K. B. Rameshkumar, B. Kumar, A. Singh, C. Anilkumar, *Ind. Crop. Prod.,* 2014, **62**, 430-436.

7 L. Kato, R. Marques Braga, I. Koch, L. Sumiko Kinoshita, *Phytochemistry,* 2002, **60**, 315-320.

8 A. Itoh, T. Kumashiro, M. Yamaguchi, N. Nagakura, Y. Mizushina, T. Nishi, T. Tanahashi, *J. Nat. Prod.,* 2005, ***68***, 848-852.

9 G. Lemieux, A. Davignon, J. Genest, *Can. Med. Assoc. J.,* 1956, **74**, 522-526.

10 K. P. Madhusudanan, S. Banerjee, S. P. S. Khanuja, S. K. Chattopadhyay, *Biomed. Chrom.*, 2008, **22**, 596-600.

11 D. S. Fabricant, N. R. Farnsworth, *Environ. Health. Persp.,* 2001, **109**, 69-75.

12 C. Alagesaboopathi, *Ethnobot. Leaflets,* 2009, **13**, 644-650.

13 P. H. Rajasree, R. Singh, C. Sankar, *International Journal of Drug Dscovery and Herbal Research,* 2013, **3**, 521-524.

14 N. Shariff, M. S. Sudarshana, S. Umesha, P. Hariprasad, *Afr. J. Biotechnol.,* 2006, **5**, 946-950.

15 K. Suresh, S. S. Babu, R. Harisaranraj, *Ethnobot. Leaflets* 2008, **77**, 586-90.

16 S. R. Deshmukh, D. S. Ashrit, B. A. Patil, *Int. J. Pharmacy. Pharma. Sci.,* 2012, **4**, 329-334.

17 S. N. Y. Fanso-Free, G. T. Furst, P. R. Srinivasan, R. L. Lichter, R. B. Nelson, J. A. Panetta, G. W. Gribble, *J. Am. Chem. Soc.,* 1979, **101**, 1549-1553.

18 R. H. Levin, J. Y. Lallemand, J. D. Roberts, *J. Org. Chem.,* 1973, **38**, 1983-1986.

19 W. E. Rosen, J. N. Shoolery, *J. Am. Chem. Soc.,* 1961, **83**, 4816-4819.

20 A. Srivastava, A. K. Tripathi, R. Pandey, R. K. Verma, M. M. Gupta, *J. Chromatogr. Sci.,* 2006, **44**, 557-560.

21 B. Hong, W. J. Li, A. Song, C. Zhao, *J. Chromatogr. Sci.*, 2013, **51**, 926-930

22 B. Hong, W. Cheng, J. Wu, C. Zhao, *Chromatographia* 2010, **72**, 841-847.

23 M. Wells, M. J. Roth, A. D. Keil, J. W. Grossenbacher, D. R.Justes, G. E.Patterson, and D. J. BarketJr J Am Soc Mass Spectrom 2008, **19**, 1419-1424.

24 H. Wang, Ni. E. Manicke, Q. Yang, L. Zheng, R. Shi, R. G. Cooks, and Z. Ouyang Anal. Chem.2011, **83**, 1197–1201

25 J. Liu, H. R. Wang, Cooks, Z. Ouyang, Anal. Chem.2011, **83**, 7608–761.

26 H. Chen; Y. Sun; A. Wortmann; H. Gu; R. Zenobi, Anal. Chem.2007, **79**, 1447–1455.

27 B. Hu, P. So, H. Chen, and Z. Yao, Anal. Chem.2011, **83**, 8201–8207.

28 Z. Takats, J. M. Wiseman, R. G. Cooks, (2005). J. Mass Spectrom.2005;40: 1261-1275

29 T. Cajka, K. Riddellova, M. Tomaniova, J. Hajslova, *Metabolomics,* 2011, **7**, 500-508.

30 R. B. Cody, J. A. Laramee, J. M. Nilles, H. D. Durst, *JEOL News,* 2005, **40**, 8-12.

31 M. Zhou, J. F. McDonald, F. M. Fernandez, *J. Am. Soc. Mass Spectrom.,* 2010, **21**, 68-75.

32 M. Curtis, M. A. Minier, P. Chitranshi, O. D. Sparkman, P. R. Jones, L. Xue, *J. Am. Soc. Mass. Spectrom.,* 2010, **21**, 1371-1381.

33 A. T. Navare, J. G. Mayoral, M. Nouzova, F. G. Noriega, F. M. Fernandez, *Anal. Bioanal. Chem.,* 2010, ***398***, 3005-3013.

34 J. M. Nilles, T. R. Connell, S. T. Stokes, H. Dupont Durst, *Propellants Explos. Pyrotech.,* 2010, **35**, 446-451.

35 F. M. Fernandez, R. B. Cody, M. D. Green, C. Y. Hampton, R. McGready, S. Sengaloundeth, N. J. White, P. N. Newton, *Chem.Med.Chem.,* 2006, **1**, 702-705.

36 J. P. Williams, V. J. Patel, R. Holland, J. H. Scrivens, *Rapid Commun. Mass Spectrom.,* 2006, **20**, 1447-1456.

37 O. P. Haefliger, N. Jeckelmann, *Rapid Commun. Mass Spectrom.,* 2007, **21**, 1361-1366.

38 T. Rothenbacher, W. Schwack, *Rapid Commun. Mass Spectrom.,* 2009, **23**, 2829-2835.

39 J. M. Nilles, T. R. Connell, H. D. Durst, *Anal. Chem.,* 2009, **81**, 6744-6749.

40 C. Y. Pierce, J. R. Barr, R. B. Cody, R. F. Massung, A. R. Woolfitt, H. Moura, Herbert A. *Chem. Comm.,* 2007, 8, 807-809.

41 E. Block, A. J. Dane, S. Thomas, R. B. Cody, *J. Agr. Food Chem.,* 2010, **58**, 4617-4625.

42 S. Edison, L. A. Lin, B. M. Gamble, J. Wong, K. Zhang, *Rapid Commun. Mass Spectrom.,* 2011, **25**, 127-129.

43 J. H Gross, "Direct analysis in real time-a critical review on DART-MS." *Anal. Bioanal. Chem.*, 2014, **1**, 63-80.

44 S. M. Lee, H. J. Kim, Y. P. Jang, *Phytochem. Analysis,* 2012, **23**, 508-512.

45 H. J. Kim, Y. P. Jang, *Phytochem. Anal.,* 2009, **20**, 372-377.

46 V. Bajpai, D. Sharma, B. Kumar, K. P. Madhusudanan, *Biomed. Chromatogr.,* 2010, **24**, 1283-1286.

47 P. Chandra, V. Bajpai, M. Srivastva, K. B. R. Kumar, B. Kumar, *Anal. Methods,* 2014*, **6,*** 4234–4239.

48 V. Bajpai, A. Singh, K. R. Arya, M. Srivastava, B. Kumar, *Food* A*ddit. Contam.* Part A, **DOI:** 10.1080/19440049.2015.1022885

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Table 1. Exact mass data for identified MIAs and its distribution in roots and leaves of six *Rauwolfia* species

S. No.	Compounds	Measured mass $[M+H]^+$	Calculated mass $[M+H]^+$	Molecular Formula	Error (ppm)	Root						Leaf					
						Rv	Rs	Rt	Rvm	Rm	Rh	Rt	Rh	Rvm	Rm	Rs	Rv
1.	Vellosiminol	295.1810	295.1815	$C_{19}H_{22}N_2O$	1.5	-	-	-	-	-	-	-	-	-	-	+	-
2.	Demethoxypurpeline	307.1890	307.1821	$C_{20}H_{23}N_2O$	3.75	+	+	-	-	-	-	-	-	-	-	+	-
3.	Tetraphyllicine	309.1970	309.1958	$C_{20}H_{25}N_2O$	2.58	-	+	-	-	-	-	-	-	-	-	+	-
4.	Sarpagine	311.1760	311.1758	$C_{19}H_{23}N_2O_2$	0.29	+	+	+	-	-	+	-	-	-	+	+	-
5.	Norajmaline	313.1920	313.1905	$C_{19}H_{25}N_2O_2$	-3.36	+	-	-	-	-	+	-	+	-	+	+	-
6.	Methylsarpagine	325.1920	325.1917	$C_{20}H_{25}N_2O_2$	0.35	-	+	+	+	-	+	-	-	-	+	-	-
7.	Ajmaline	327.2070	327.2041	$C_{20}H_{27}N_2O_2$	4.57	+	+	+	+	-	-	-	-	-	-	-	-

8.	Acetylnortetraphyllicine	337.1920	337.1897	$C_{21}H_{25}N_2O_2$	3.4	+	+	-	-	-	-	-	-	-	-	-	-
9.	Sandwicolidine	341.2230	341.2234	$C_{21}H_{28}N_2O_2$	1.52	-	+	+	+	-	-	-	-	-	-	-	-
10.	Ajmalinol	343.2210	343.2209	$C_{21}H_{30}N_2O_2$	0.69	-	+	+	+	-	-	-	-	-	-	-	-
11.	Vomilenine	351.1710	351.1717	$C_{21}H_{23}N_2O_3$	2.51	-	+	-	-	+	+	-	+	-	+	-	+
12.	Ajmalicine	353.1870	353.1872	$C_{21}H_{25}N_2O_3$	2.19	-	-	-	+	+	-	+	+	-	+	-	-
13.	Yohimbine	355.2020	355.2024	$C_{23}H_{27}N_2O_3$	0.92	-	-	-	+	-	-	+	+	+	+	-	-
14.	Demethoxyreserpiline	383.1970	383.1988	$C_{22}H_{27}N_2O_4$	4.59	+	-	-	+	-	-	+	-	-	+	+	-
15.	Darcyriberine	411.1920	411.1913	$C_{23}H_{27}N_2O_5$	-1.62	-	+	-	-	+	+	+	+	+	-	-	-
16.	Reserpiline	413.2080	413.2092	$C_{23}H_{29}N_2O_5$	3.92	+	-	-	-	+	+	+	+	+	+	-	-
17.	Reserpine	609.2810	609.2841	$C_{23}H_{41}N_2O_9$	4.76	+	-	-	-	-	-	-	-	-	-	-	-

(+): detected, (-): not detected, Rv: *R. verticillata,* Rs*: R. serpentina* Rt: *R. tetraphylla,* Rvm: *R. vomitoria* Rm: *R. micrantha* Rh: *R. hookeri.*

Fig.1 Comparative DART-MS fingerprint spectra of roots of six *Rauwolfia* species in positive mode.

Fig.2 Comparative DART-MS fingerprint spectra of leaves of six *Rauwolfia* species in positive mode.

Fig. 3 (%) Ionization of compounds in six *Rauwolfia* species: (A) root; and (B) leaves. (Rv: *R. verticillata*, Rs: *R. Serpentina,* Rt: *R. tetraphylla*, Rvm: *R. vomitoria* Rm: *R. micrantha* Rh: *R. Hookeri*).

Fig. 4 PC1 *vs.* PC2 plot showing distinct discrimination among the *R. hookeri, R. micrantha, R. serpentina, R. tetraphylla, R. verticillata* and *R. vomitoria* in roots (A) and leaves (B) on the basis of marker peaks. Blue and red spots represent training and validated data sets respectively.

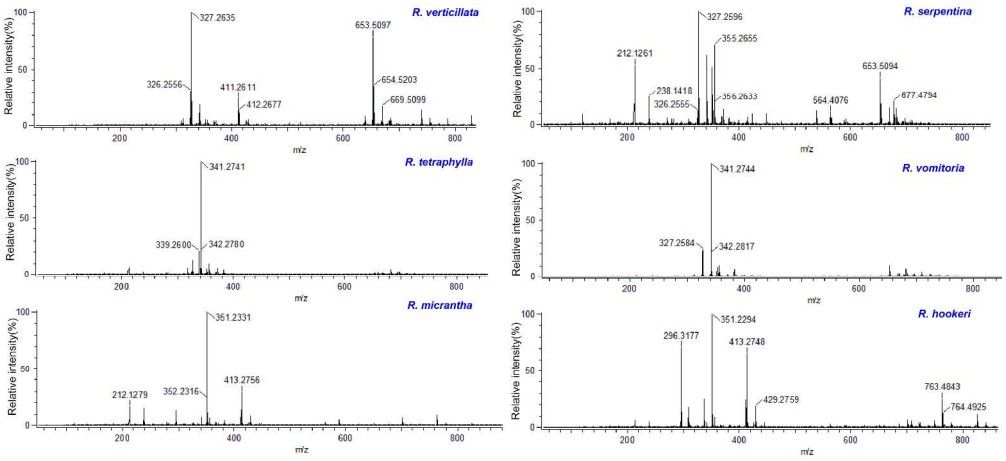

Fig.1 Comparative DART-MS fingerprint spectra of roots of six Rauwolfia species in positive mode.
395x180mm (300 x 300 DPI)

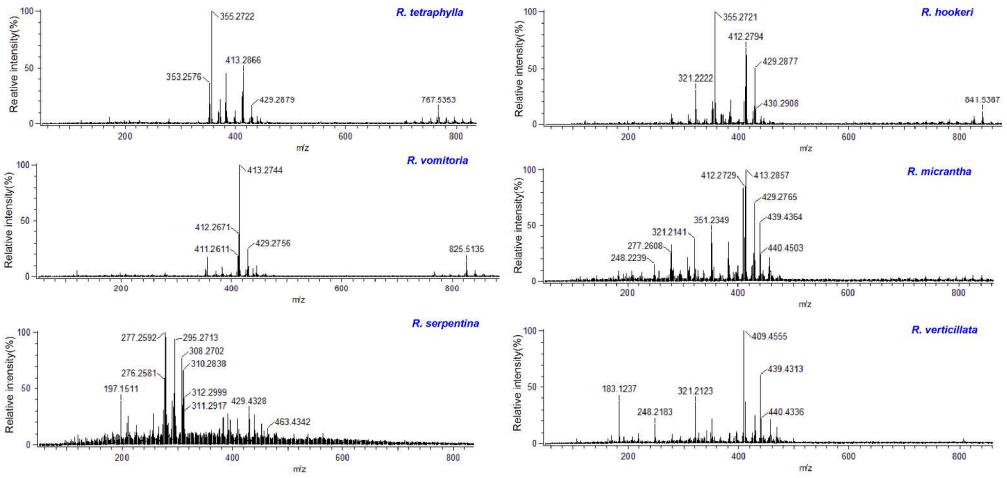

Fig.2 Comparative DART-MS fingerprint spectra of leaves of six Rauwolfia species in positive mode.
400x188mm (300 x 300 DPI)

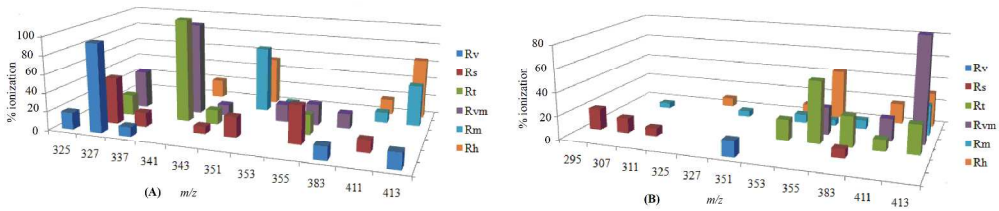

Fig. 3 (%) Ionization of compounds in six Rauwolfia species: (A) root; and (B) leaves.
355x77mm (300 x 300 DPI)

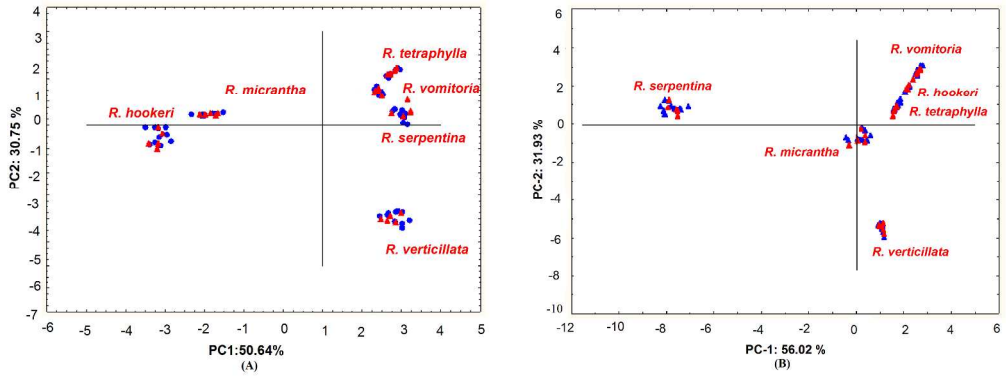

Fig. 4 PC1 vs. PC2 plot showing distinct discrimination among the R. hookeri, R. micrantha, R. serpentina, R. tetraphylla, R. verticillata and R. vomitoria in roots (A) and leaves (B) on the basis of marker peaks. Blue and red spots represent training and validated data sets respectively.
343x129mm (300 x 300 DPI)