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Profiling of *Piper betle* Linn. cultivars by direct analysis in real time mass spectrometric technique

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ABSTRACT: *Piper betle* Linn. is a traditional plant associated with the Asian and southeast Asian cultures. Its use is also recorded in folk medicines in these regions. Several of its medicinal properties have recently been proven. Phytochemical analysis showed the presence of mainly terpenes and phenols in betel leaves. These constituents vary in the different cultivars of *Piper betle*. In this paper we have attempted to profile eight locally available betel cultivars using the recently developed mass spectral ionization technique of direct analysis in real time (DART). Principal component analysis has also been employed to analyze the DART MS data of these betel cultivars. The results show that the cultivars of *Piper betle* could be differentiated using DART MS data. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: DARTMS; *Piper betle* L; cultivars; profiling; PCA

Introduction

Piper betle Linn. (betel vine) is a tropical plant closely related to the common pepper and belongs to the family Piperaceae (Gunther, 1952). It is extensively grown in India, Sri Lanka, Malaysia, Thailand, Taiwan and other southeast Asian countries and has a long history of over 2000 years (Guha, 2006). Chewing of betel quid has been practised since ancient times and is prevalent in India, other Asian countries, the western pacific and among migrated communities in Africa, Europe and North America (Gupta and Ray, 2004). Betel quid generally consists of betel leaf (from the *Piper betle* L. vine), areca nut (from the *Areca catechu* tree), catechu (a tannin-rich powder) and slaked lime (calcium hydroxide), to which tobacco is often added. There have been many reports that chewing betel quid causes oral cancer (IARC, 1985; Sankaranarayanan, 1990; Moore *et al*., 2000; Avon, 2004).

Betel leaf is an integral part of Asian, and, of course, Indian history. Its utility against various diseases can be traced in the ancient vedic literature. An exhaustive list of its properties and uses is presented in a recent compilation (Warrier *et al*., 1995). *Piper betle* is aromatic, stimulant, carminative, astringent and antiseptic. Experimentally *Piper betle* leaves have been found to have diverse pharmacological actions (Nadkarni, 1976), such as anti-inflammatory (Sarkar *et al*., 2008), anti-oxidant (Choudhary and Kale, 2002), radio-protective (Bhattacharya *et al*., 2005), and anti-allergic activities (Wirotesangthong *et al*., 2008). They have also shown anti-microbial (Shitut *et al*., 1999), anti-fungal (Trakranrungsie *et al*., 2008), anti-amoebic (Sawangjaroen *et al*., 2006), hepatoprotective (Manigauha *et al*., 2009) anti-fertility (Sarkar *et al*., 2000) and anti-platelet activities (Lei *et al*., 2003).

The chemical constituents of betel essential oil consist of mainly terpenes and phenols (Atal *et al*., 1975; Balasubrahmanyam and Rawat, 1990; Rimando *et al*., 1986; Mohottalage *et al*., 2009). The characteristic flavour of betel is due to the betel phenols. The terpenoids include 1,8-cineole, cadinene, camphene, caryophylline, limonene, pinene, etc. Chavicol, allyl pyrocatechol, carvacrol, safrole, eugenol and chavibetol are the major phenols found in *Piper betle*. Their acetates are also commonly found.

Since the crop of *Piper betle* requires vegetative propagation and is widely cultivated, it is claimed to have 100 cultivars (landraces) based on regional and organoleptic considerations (Verma *et al*., 2004). Only isolated reports exist on the chemical profiling of these cultivars (Rawat *et al*., 1987; Rawat *et al*., 1989).

Direct analysis in real time (DART) is a new ambient ionization technique (Cody *et al*., 2005). Using a helium plasma DART ionizes atmospheric water and generates water clusters which in turn ionize the sample held in the gas stream. The resulting spectra are relatively clean and simple. As DART ion source can ionize molecules directly from the surface, plant products can be analyzed directly without sample preparation (Haefliger and Jeckelmann, 2007; Madhusudanan *et al*., 2008; Kim and Jang, 2009). In view of the ease with which natural products can be analyzed by DARTMS, it was thought that DART MS could be a suitable tool for the chemical profiling of the different landraces of *Piper betle* leaves. Locally available cultivars, namely Bangla (**1**), Desawari (**2**), Deshi (**3**), J. Green (**4**), J. White (**5**), Kalkatiya (**6**), Mahoba (**7**) and Saufia (**8**), of *Piper betle* were analyzed by DARTMS and the results are presented here.

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Abbreviations used: DART, direct analysis in real time; PCA, principal component analysis; TOF MS, time-of-flight mass spectrometry.

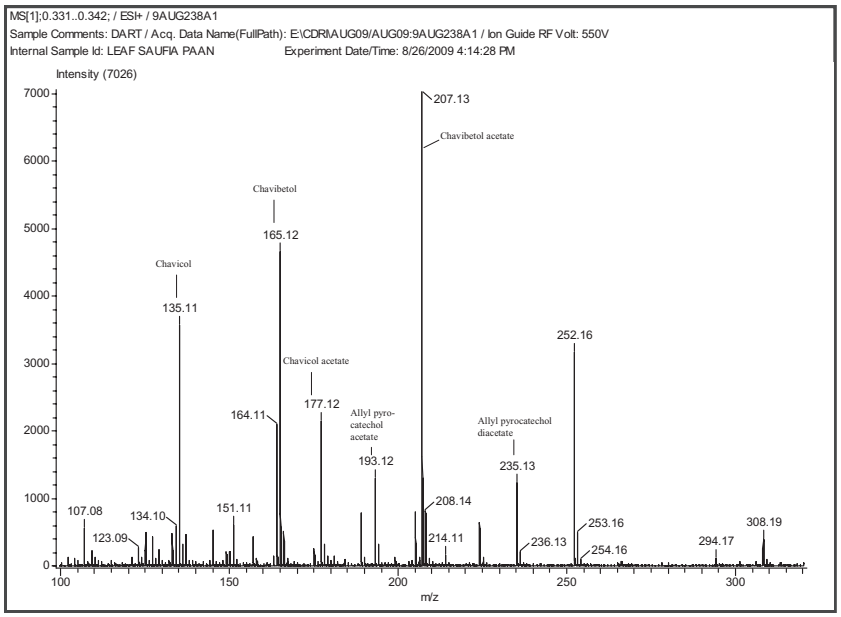

Figure 1. DART mass spectrum *Piper betle* leaf of the cultivars Saufia (**8**).

Table 1. Exact mass data from the DART mass spectra of *Piper betle* leaves

Molecular weight	Measured mass	Calculated mass	Molecular formula	Error (mmu)	Remarks
134	135.08128	135.08099	$C_9H_{11}O$	0.29	Chavicol
150	151.07640	151.07590	$C_9H_{11}O_2$	0.50	Allylpyrocatechol
164	165.09242	165.09155	$C_{10}H_{13}O_2$	0.87	Chavibetol
165	166.08864	166.08680	$C_9H_{12}NO_2$	1.84	Phenyl alanine
174	175.07764	175.07590	$C_{11}H_{11}O_2$	1.74	Unknown
176	177.09109	177.09155	$C_{11}H_{13}O_2$	−0.46	Chavicol acetate
192	193.08772	193.08647	$C_{11}H_{13}O_3$	1.25	Allylpyrocatechol acetate
206	207.10140	207.10212	$C_{12}H_{15}O_3$	−0.72	Chavibetol acetate
234	235.09649	235.09703	$C_{13}H_{15}O_4$	−0.54	Allylpyrocatechol diacetate
251	252.12287	252.12358	$C_{13}H_{18}NO_4$	−0.71	Unknown

Experimental

The eight cultivars of betel leaves were collected from the local market. The leaves were washed and wiped dry. 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 heater was set to 300°C. 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. Freshly cut pieces of betel leaf were positioned in the gap between the DART source and mass spectrometer for measurements. Data acquisition was from *m/z* 10 to 1050. 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. *m*-Nitrobenzyl alcohol was also used for calibration. The mass calibration was accurate to within ±0.002 u. Using the Mass Center software, the elemental composition could be determined on selected peaks. PCA analysis was carried out using Minitab 14 statistical analysis software (Trial Version).

Results and Discussion

A representative DART mass spectrum of *Piper betle* leaf is given in Fig. 1. The DART mass spectra did not show peaks attributable to terpenes except a small peak at *m/z* 205 corresponding to sesquiterpenes. However, peaks were observed at *m/z* values corresponding to many of the reported phenols and their acetates in *Piper betle* leaf. Accordingly, peaks at *m/z* 135, 151, 165, 177, 193, 207 and 235 could be due to chavicol, allylpyrocatechol, chavibetol, chavicol acetate, allylpyrocatechol acetate, chavibetol acetate and allylpyrocatechol diacetate, respectively. The exact mass values measured using the DART TOF MS are given in Table 1. Since some of the phenols have the same molecular weight it was not possible to distinguish them from DART mass spectra alone. The peak at *m/z* 151 could be due to allylpyrocatechol or carvacrol. These two molecules have different molecular formulae and hence could be differentiated based on their exact mass values. In the present study the formula corresponded to allyl pyrocatechol. The peak at *m/z* 165 could be due to eugenol or chavibetol. Since both have the same molecular formula, a distinction could not be made.

There were differences in the spectra of the different cultivars, as shown in Table 2. A peak at *m/z* 135 corresponding to chavicol was seen only in the cultivars Saufia. The data shows that allylpyrocatechol is present in all cultivars except Desawari, whereas chavibetol is not present in Deasawari and Mahoba cultivars. It also shows that chavicol acetate is present only in Bangla and Saufia cultivars. The peaks at *m/z* 193, 207, 235 and 252 are common to all cultivars.

It is not easy to identify and differentiate all the components independently based on their mass spectral data. MS-based chemical profiling generates complex data sets which need sophisticated software to enable interpretation. Visualization is a key aspect as the data contained a number of variables. Multivariate analysis such as principal component analysis (PCA) can be used to reduce the dimensionality and to allow independent classification of cases. PCA groups the samples solely on information on the measured data and does not need any extra knowledge about the sample, and therefore can be used to summarize and visualize the structure of the data. The mass spectral data (five sets) for all the eight *Piper betle* leaves were subjected to PCA using 14 variables (abundances of ions at *m/z* 104, 115, 123, 150, 151, 165, 175, 193, 207, 235, 252, 308, 324, 352). The PCA score plot clearly brings out the relationship among all the betel data and all the eight sets of betel cultivars are clearly separated (Fig. 2). It seems that Saufia and Bangla are entirely different from all other cultivars, whereas pairs of J. White and Desawari are much closer to each other. Similarly, Mahoba and Kalkatiya are similar but Deshi appears between J. White/Desawari and Mahoba/Kalkatiya pairs. It is evident from this study that PCA effectively served the purpose and all the betel cultivars could be differentiated by this method.

Table 2. DART mass spectral data of Bangla (**1**), Desawari (**2**), Deshi (**3**), J. Green (**4**), J. White (**5**), Kalkatiya (**6**), Mahoba (**7**) and Saufia (**8**) cultivars of *Piper betle* leaves

m/z	*Piper betle* cultivars							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
104	—[a]	×	—	—	×	×	—	—
118	×	×	—	—	×	—	—	—
132	×	×	—	—	×	—	—	—
135	—	—	—	—	—	—	—	×
151	×	—	×	×	×	×	×	×
163	×	—	—	×	×	×	×	×
165	×	—	×	×	×	×	—	×
166	—	×	—	—	×	—	—	—
175	×	—	×	×	×	×	×	—
177	×	—	—	—	—	—	—	×
193	×	×	×	×	×	×	×	×
205	×	—	—	×	×	×	—	×
207	×	×	×	×	×	×	×	×
235	×	×	×	×	×	×	×	×
252	×	×	×	×	×	×	×	×

[a] —, Absent; ×, present.

Conclusion

The DART MS of the leaves *Piper betle* could be recorded without any sample preparation. The abundances of the characteristic betel phenols were different in the eight cultivars. Principal component analysis showed the expected grouping of the cultivars.

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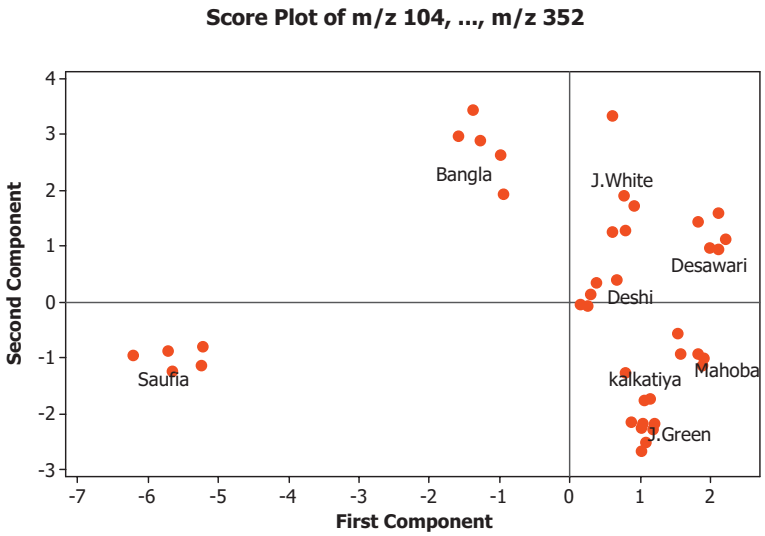

Figure 2. PCA score plot of the abundances of the various ions in the DART mass spectra of the leaves of various betel cultivars.

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